

Treatments Supplement

Ana Jimenez
Colgate University
Department of Biology

Sahil Lalwani
Colgate University
Department of Mathematics

William Cipolli III*
Colgate University
Department of Mathematics
will@cipolli.com

1 Introduction

The models are fit in R (R Core Team, 2020) and the mixed effects models are fit using the `lmerTest` package (Kuznetsova et al., 2017) for R. Best-subset model selection according to the Bayesian information criterion (BIC) (Schwarz et al., 1978) was completed via complete enumeration (Morgan and Tatar, 1972) for each model. BIC penalizes false positives more than false negatives when there are eight or more observations.

We adjusted the p-values in the MANOVA analyses and within each model reported using the approach in Benjamini and Hochberg (1995) instead of the overly conservative Bonferroni adjustment (Dunn, 1961). This approach allows for the control of the expected proportion of false discoveries instead of the probability of making at least one false discovery, thus preserving power.

In Table 0, we provide a condensed, succinct table showing the overall research design and accompanying variables of interest.

1.1 Overall MANOVA

We completed a repeated measures multivariate analysis of variance (MANOVA) which is a multivariate extension of a regular repeated measures analysis of variance (ANOVA).

*All R Code and data can be provided by request to will@cipolli.com

	DF	Pillai	Approx F.	DF (num)	DF (denom)	p
Age.Class	1.0000	0.1861	3.5432	8.0000	124.0000	0.0070
treatment	3.0000	0.3441	2.0407	24.0000	378.0000	0.0106
Size.Class	1.0000	0.1476	2.6846	8.0000	124.0000	0.0218
Age.Class:treatment	3.0000	0.1728	0.9628	24.0000	378.0000	0.5154
Age.Class:Size.Class	1.0000	0.0902	1.5373	8.0000	124.0000	0.2113
treatment:Size.Class	3.0000	0.2322	1.3213	24.0000	378.0000	0.2113
Age.Class:treatment:Size.Class	2.0000	0.1192	0.9900	16.0000	250.0000	0.5154

Table 1: Overall MANOVA.

The MANOVA results in Table 1 show significance for the multivariate analysis in age class, size class, and treatment so we probe each dependent variable separately using ANOVA below.

Remark: We added 1 to Proton Leak /20K and Spare Respiratory Capacity/20K to avoid issues when performing the log-transform where measurements were zero.

1.2 ANOVA Results

The repeated measures ANOVA results below suggest significant differences in Non-Glycolytic Acidification/20K across age class and treatment; Total Glycolysis/20K across treatment; Glycolytic capacity/20K across treatment and age class; Basal OCR/20K across treatment, age class, interaction between treatment group*age class and interaction between treatment*age class*size class; Proton Leak/20K across treatment, age class and interaction between treatment group*age class; Maximal Respiration/20K across treatment, age class, interaction between treatment group*age class and interaction between treatment*age class*size class; Spare Respiratory Capacity/20K across treatment, age class and interaction between age class*size class; Non-Mito Respiration/20K across treatment, age class and interaction between treatment group*age class.

	Chi-squared	DF	p
(Intercept)	476.6876	1.0000	<0.0001
treatment	24.1837	3.0000	0.0001
Age.Class	14.2207	1.0000	0.0004
Size.Class	1.0873	1.0000	0.5660
treatment:Age.Class	3.2564	3.0000	0.5660
treatment:Size.Class	2.0758	3.0000	0.6364
Age.Class:Size.Class	0.6308	1.0000	0.5694
treatment:Age.Class:Size.Class	0.8413	3.0000	0.8396

Table 2: ANOVA analysis for log-transformed Non-Glycolytic Acidification/20K.

	Chi-squared	DF	p
(Intercept)	903.7802	1.0000	<0.0001
treatment	13.6510	3.0000	0.0137
Age.Class	1.9565	1.0000	0.4317
Size.Class	0.2249	1.0000	0.9183
treatment:Age.Class	0.7161	3.0000	0.9183
treatment:Size.Class	4.0462	3.0000	0.5130
Age.Class:Size.Class	0.0105	1.0000	0.9183
treatment:Age.Class:Size.Class	0.7324	3.0000	0.9183

Table 3: ANOVA analysis for log-transformed Glycolysis/20K.

	Chi-squared	DF	p
(Intercept)	1322.9985	1.0000	<0.0001
treatment	15.3700	3.0000	0.0061
Age.Class	5.8055	1.0000	0.0426
Size.Class	0.2036	1.0000	0.8691
treatment:Age.Class	0.8862	3.0000	0.9349
treatment:Size.Class	4.7065	3.0000	0.3892
Age.Class:Size.Class	0.5537	1.0000	0.7309
treatment:Age.Class:Size.Class	0.4258	3.0000	0.9349

Table 4: ANOVA analysis for log-transformed Glycolytic Capacity/20K

	Chi-squared	DF	p
(Intercept)	315.8041	1.0000	<0.0001
treatment	48.8006	3.0000	<0.0001
Age.Class	9.1192	1.0000	0.0051
Size.Class	0.6845	1.0000	0.4080
treatment:Age.Class	19.7241	3.0000	0.0005
treatment:Size.Class	2.9455	3.0000	0.4080
Age.Class:Size.Class	1.6760	1.0000	0.2606
treatment:Age.Class:Size.Class	10.3468	3.0000	0.0253

Table 5: ANOVA analysis for log-transformed Basal OCR/20K.

	Chi-squared	DF	p
(Intercept)	245.9741	1.0000	<0.0001
treatment	37.1106	3.0000	<0.0001
Age.Class	6.5332	1.0000	0.0212
Size.Class	1.7814	1.0000	0.2080
treatment:Age.Class	13.2019	3.0000	0.0113
treatment:Size.Class	1.2654	3.0000	0.7374
Age.Class:Size.Class	2.1868	1.0000	0.1856
treatment:Age.Class:Size.Class	8.2529	3.0000	0.0657

Table 6: ANOVA analysis for log-transformed Proton Leak/20K.

	Chi-squared	DF	p
(Intercept)	428.8734	1.0000	<0.0001
treatment	19.6483	3.0000	0.0005
Age.Class	13.3988	1.0000	0.0005
Size.Class	0.0749	1.0000	0.7843
treatment:Age.Class	32.8512	3.0000	<0.0001
treatment:Size.Class	1.4600	3.0000	0.7843
Age.Class:Size.Class	3.1323	1.0000	0.1023
treatment:Age.Class:Size.Class	17.4693	3.0000	0.0009

Table 7: ANOVA analysis for log-transformed Maximal Respiration/20K.

	Chi-squared	DF	p
(Intercept)	323.2038	1.0000	<0.0001
treatment	9.5943	3.0000	0.0447
Age.Class	13.2619	1.0000	0.0011
Size.Class	0.5225	1.0000	0.5369
treatment:Age.Class	8.0619	3.0000	0.0716
treatment:Size.Class	0.9241	3.0000	0.8196
Age.Class:Size.Class	5.2992	1.0000	0.0447
treatment:Age.Class:Size.Class	5.9090	3.0000	0.1548

Table 8: ANOVA analysis for log-transformed Spare Respiratory Capacity/20K.

	Chi-squared	DF	p
(Intercept)	402.4773	1.0000	<0.0001
treatment	32.0535	3.0000	<0.0001
Age.Class	15.9779	1.0000	0.0002
Size.Class	4.0624	1.0000	0.0585
treatment:Age.Class	14.0996	3.0000	0.0055
treatment:Size.Class	1.5810	3.0000	0.6637
Age.Class:Size.Class	1.4145	1.0000	0.2678
treatment:Age.Class:Size.Class	8.4401	3.0000	0.0585

Table 9: ANOVA analysis for log-transformed Non-Mito Respiration/20K.

2 Non-Glycolytic Acidification/20K

2.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	1.8271	0.3423	147.4656	5.3375	<0.0001
Age.ClassOld	0.6044	0.2491	307.0540	2.4262	0.0712
SexM	0.0076	0.0989	122.9447	0.0767	0.9390
Size.ClassL	-0.2376	0.1538	307.0680	-1.5448	0.3703
breed.lifespan	0.0584	0.0281	132.9699	2.0757	0.1434
treatmentMetformin	0.2417	0.2196	342.5091	1.1002	0.5468
treatmentRapamycin	0.4656	0.1471	318.4991	3.1652	0.0102
treatmentResveratrol	0.4859	0.1396	310.1316	3.4816	0.0051
Age.ClassOld:treatmentMetformin	-0.3811	0.4531	351.8400	-0.8411	0.6560
Age.ClassOld:treatmentRapamycin	-0.3177	0.3068	327.4446	-1.0357	0.5468
Age.ClassOld:treatmentResveratrol	-0.3826	0.2733	310.1316	-1.4001	0.4178
Size.ClassL:treatmentMetformin	-0.0991	0.2440	339.8894	-0.4061	0.7822
Size.ClassL:treatmentRapamycin	-0.1184	0.1753	316.0240	-0.6753	0.6922
Size.ClassL:treatmentResveratrol	-0.1745	0.1695	310.6440	-1.0300	0.5468
Age.ClassOld:Size.ClassL	-0.0889	0.3332	315.8632	-0.2668	0.8363
Age.ClassOld:Size.ClassL:treatmentMetformin	-0.3652	0.6098	349.9589	-0.5989	0.7067
Age.ClassOld:Size.ClassL:treatmentRapamycin	0.1560	0.3980	320.4113	0.3921	0.7822
Age.ClassOld:Size.ClassL:treatmentResveratrol	0.2539	0.3730	310.2374	0.6806	0.6922

Table 10: Summary of the log-transformed full model for Non-Glycolytic Acidification/20K.

2.1.1 Post-hoc Analysis

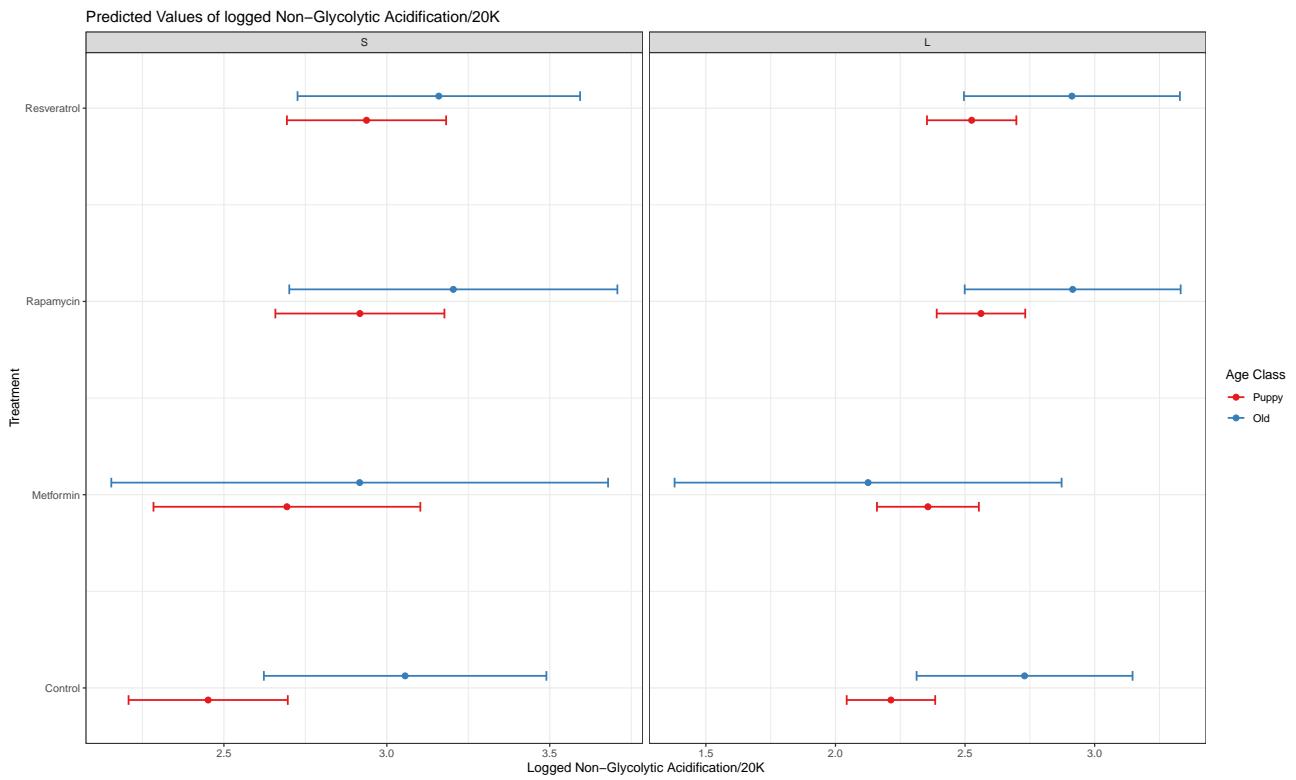


Figure 1: Marginal means for the full log transformed model for Non Glycolytic Acidification/20K.

	Treatment	Age Class	Size Class	Est.	Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	2.4517		0.1246	315.7389	2.2065	2.6970
2	Metformin	Puppy	S	2.6934		0.2092	429.3139	2.2822	3.1046
3	Rapamycin	Puppy	S	2.9174		0.1324	345.1754	2.6570	3.1778
4	Resveratrol	Puppy	S	2.9377		0.1246	315.7389	2.6924	3.1829
5	Control	Old	S	3.0562		0.2212	290.8541	2.6208	3.4915
6	Metformin	Old	S	2.9167		0.3895	430.5972	2.1511	3.6824
7	Rapamycin	Old	S	3.2041		0.2570	363.8473	2.6986	3.7096
8	Resveratrol	Old	S	3.1595		0.2212	290.8541	2.7242	3.5949
9	Control	Puppy	L	2.2141		0.0871	301.7859	2.0426	2.3856
10	Metformin	Puppy	L	2.3567		0.1004	375.6005	2.1593	2.5540
11	Rapamycin	Puppy	L	2.5614		0.0871	301.7859	2.3899	2.7328
12	Resveratrol	Puppy	L	2.5255		0.0880	306.8748	2.3523	2.6986
13	Control	Old	L	2.7296		0.2126	308.3328	2.3114	3.1479
14	Metformin	Old	L	2.1258		0.3815	427.6479	1.3760	2.8757
15	Rapamycin	Old	L	2.9152		0.2126	308.3328	2.4969	3.3335
16	Resveratrol	Old	L	2.9123		0.2126	308.3328	2.4940	3.3305

Table 11: Summary of the marginal means for the full log transformed model for Non-Glycolytic Acidification/20k.

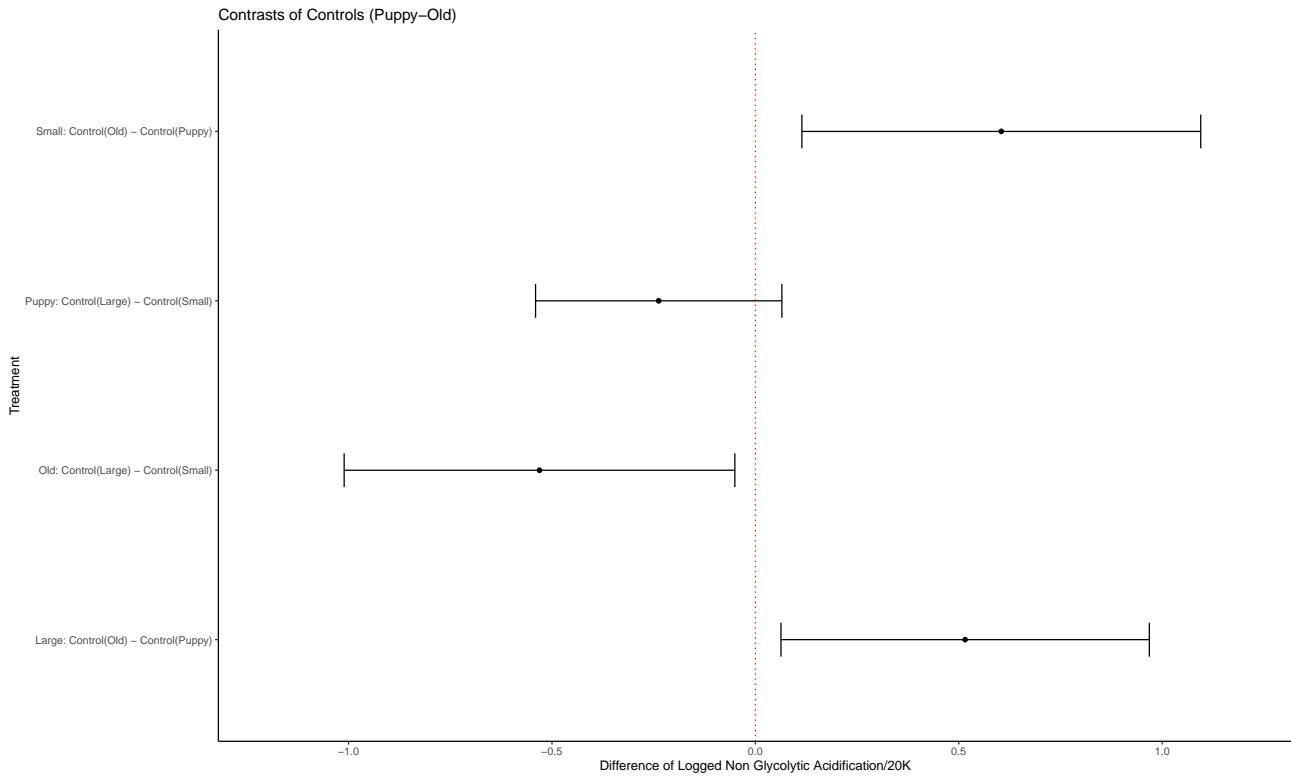


Figure 2: Pairwise treatment contrasts for the full log transformed model for Non Glycolytic Acidification/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	0.5155	0.2301	306.7930	2.2408	0.0406	0.0628	0.9682
2	Small: Control(Old) - Control(Puppy)	0.6044	0.2491	305.6254	2.4262	0.0406	0.1142	1.0947
3	Puppy: Control(Large) - Control(Small)	-0.2376	0.1538	305.6395	-1.5448	0.1234	-0.5404	0.0651
4	Old: Control(Large) - Control(Small)	-0.5307	0.2439	282.2510	-2.1754	0.0406	-1.0109	-0.0505

Table 12: Summary of the pairwise control contrast for the full log transformed model for Non-Glycolytic Acidification/20k.

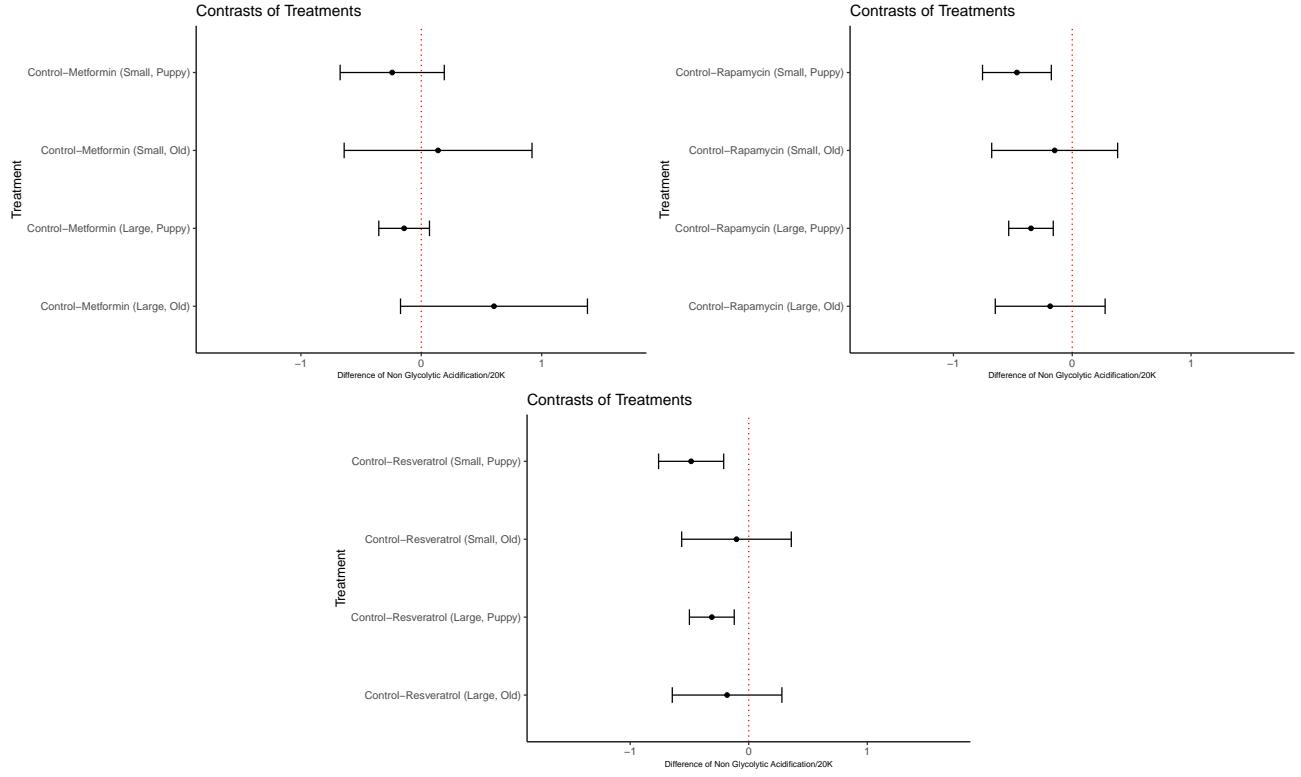


Figure 3: Pairwise treatment contrasts for the full log transformed model for Non Glycolytic Acidification/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control-Metformin (Small, Puppy)	-0.2417	0.2199	341.3699	-1.0990	0.4672	-0.6742	0.1909
2	Control-Metformin (Large, Puppy)	-0.1426	0.1070	320.0622	-1.3328	0.3671	-0.3530	0.0679
3	Control-Metformin (Small, Old)	0.1395	0.3967	354.3278	0.3515	0.7254	-0.6408	0.9197
4	Control-Metformin (Large, Old)	0.6038	0.3948	347.4337	1.5296	0.3049	-0.1726	1.3802
5	Control-Rapamycin (Small, Puppy)	-0.4656	0.1471	317.1535	-3.1644	0.0051	-0.7551	-0.1761
6	Control-Rapamycin (Large, Puppy)	-0.3473	0.0953	308.7244	-3.6457	0.0034	-0.5347	-0.1598
7	Control-Rapamycin (Small, Old)	-0.1479	0.2694	328.7178	-0.5490	0.7000	-0.6779	0.3821
8	Control-Rapamycin (Large, Old)	-0.1856	0.2349	308.7244	-0.7899	0.5834	-0.6479	0.2767
9	Control-Resveratrol (Small, Puppy)	-0.4859	0.1396	308.7244	-3.4816	0.0034	-0.7606	-0.2113
10	Control-Resveratrol (Large, Puppy)	-0.3114	0.0961	310.3296	-3.2401	0.0051	-0.5005	-0.1223
11	Control-Resveratrol (Small, Old)	-0.1033	0.2349	308.7244	-0.4398	0.7204	-0.5656	0.3590
12	Control-Resveratrol (Large, Old)	-0.1826	0.2349	308.7244	-0.7774	0.5834	-0.6449	0.2797

Table 13: Summary of the pairwise treatment contrasts for the full log transformed model for Non Glycolytic Acidification/20K.

2.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	2.5748	0.1052	181.2277	24.4854	<0.0001
Age.ClassOld	0.4764	0.1247	133.1172	3.8205	0.0003
SexM	0.0108	0.0977	124.5774	0.1104	0.9123
Size.ClassL	-0.3970	0.1028	129.4073	-3.8614	0.0003
treatmentMetformin	0.0995	0.0893	337.2146	1.1137	0.3106
treatmentRapamycin	0.3431	0.0726	321.4503	4.7279	<0.0001
treatmentResveratrol	0.3262	0.0713	318.8699	4.5781	<0.0001

Table 14: Summary of the log-transformed best subsets model for Non-Glycolytic Acidification/20K.

2.2.1 Post-hoc Analysis

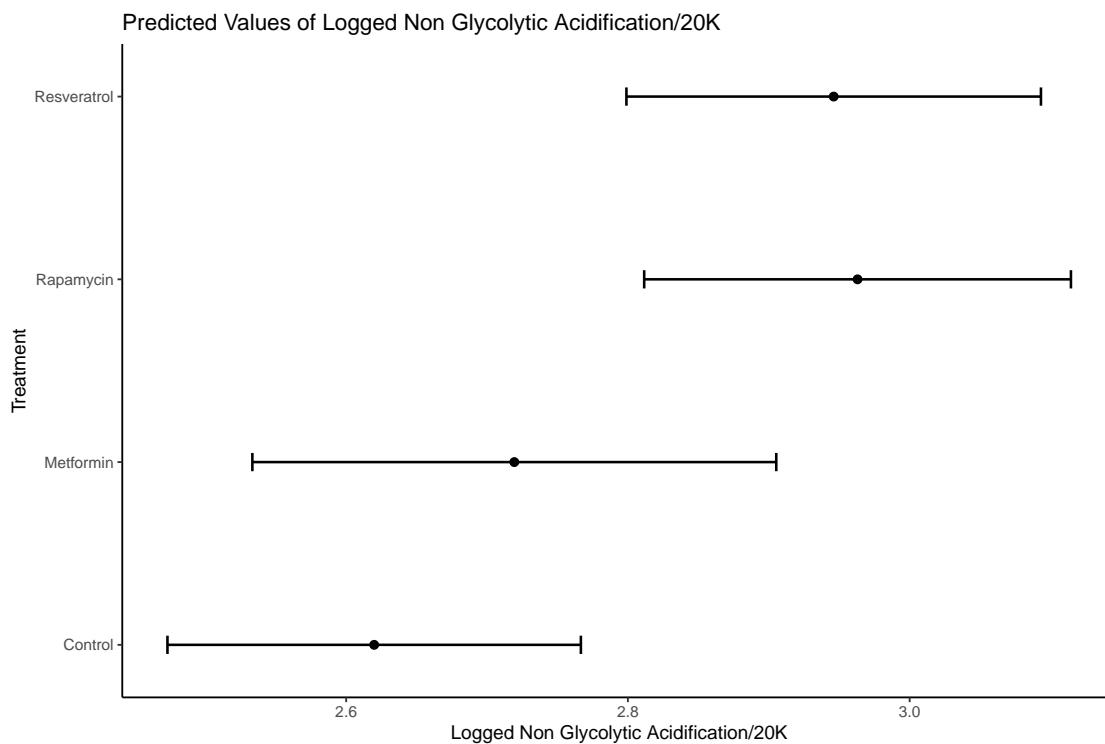


Figure 4: Marginal means for the log-transformed best subsets model for Non-Glycolytic Acidification/20K.

	Treatment	Est. Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	2.6199	0.0749	251.6977	2.4724	2.7673
2	Metformin	2.7194	0.0949	389.1831	2.5327	2.9060
3	Rapamycin	2.9630	0.0773	271.0883	2.8108	3.1152
4	Resveratrol	2.9461	0.0751	253.2983	2.7982	3.0939

Table 15: Summary of the marginal means for the log-transformed best subsets model for Non-Glycolytic Acidification/20K.

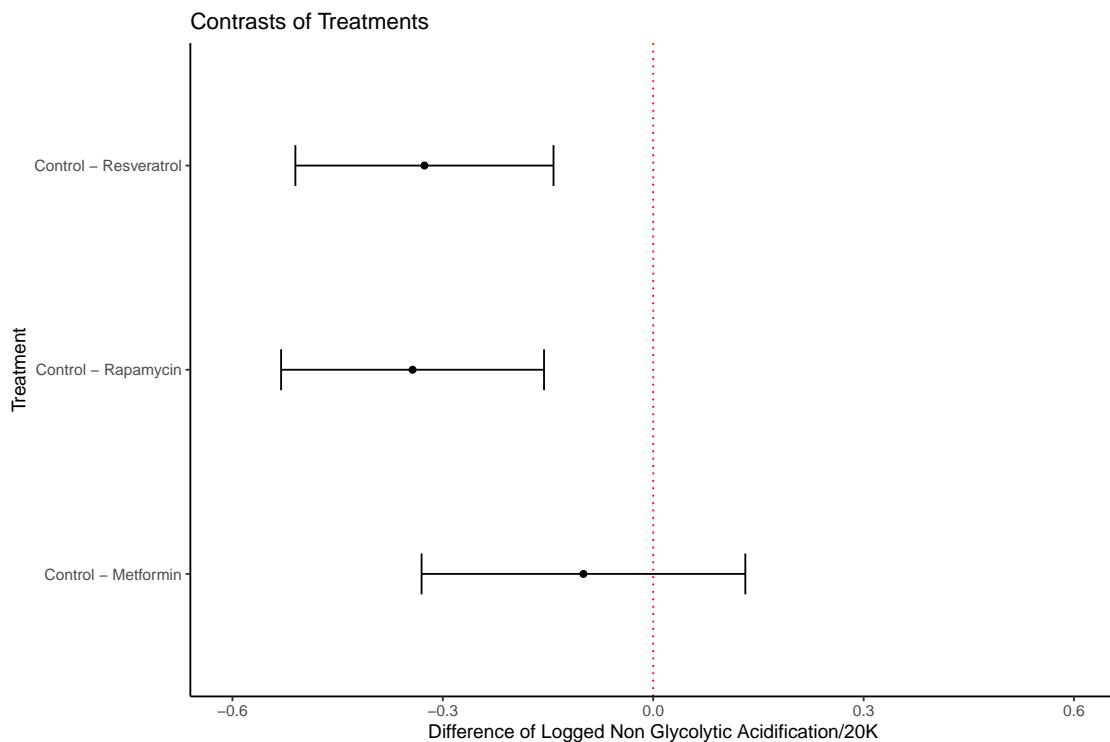


Figure 5: Pairwise treatment contrasts for the log-transformed best subsets model for Non-Glycolytic Acidification/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control - Metformin	-0.0995	0.0894	336.9715	-1.1130	0.6818	-0.3303	0.1313
2	Control - Rapamycin	-0.3431	0.0726	321.1837	-4.7275	<0.0001	-0.5306	-0.1557
3	Control - Resveratrol	-0.3262	0.0713	318.5998	-4.5780	<0.0001	-0.5102	-0.1422

Table 16: Summary of the pairwise treatment contrasts for the log-transformed best subsets model for Non-Glycolytic Acidification/20K.

2.3 Analysis

A log transform of non-glycolytic acidification/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that size class, age class and treatment with drugs Rapamycin and Resveratrol are significantly associated with non-glycolytic acidification/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 2.5748 + 0.4764X_{1i} + 0.0108X_{2i} - 0.3970X_{3i} + 0.0995X_{4i} + 0.3431X_{5i} + 0.3262X_{6i} + \epsilon_i.$$

Where

$$Y_i = \text{Non-glycolytic acidification/20K}$$

$$\alpha_i = \text{The random intercept for dog } i$$

$$X_{1i} = \text{Age class (1= Old, 0= Puppy)}$$

$$X_{2i} = \text{Sex (1=Male, 0=Female)}$$

$$X_{3i} = \text{Size class (1=Large, 0=Small)}$$

$$X_{4i} = \text{treatment group (1= Metformin, 0= Other)}$$

$$X_{5i} = \text{treatment group (1= Rapamycin, 0= Other)}$$

$$X_{6i} = \text{treatment group (1= Resveratrol, 0= Other)}$$

for each observation $i = 1, 2, \dots, n$.

To be interpretable, in terms of the response variable, we solve the regression equation for Y_i .

$$\begin{aligned} Y_i &= e^{\alpha_i} e^{2.5748} e^{0.4764X_{1i}} e^{0.0108X_{2i}} e^{-0.3970X_{3i}} e^{0.0995X_{4i}} e^{0.3431X_{5i}} e^{0.3262X_{6i}} e^{\epsilon_i} \\ &= e^{\alpha_i} (13.129) (1.6103)^{X_{1i}} (1.0109)^{X_{2i}} (0.6723)^{X_{3i}} (1.1046)^{X_{4i}} (1.4093)^{X_{5i}} (1.3857)^{X_{6i}} e^{\epsilon_i} \end{aligned}$$

- We expect a 61.03% increase in non-glycolytic acidification/20K for old dogs compared to puppies
- We expect a 1.09% increase in non-glycolytic acidification/20K for male dogs compared to female dogs
- We expect a 32.77% increase in non-glycolytic acidification/20K for small breed dogs compared to large breed dogs

- We expect a 10.46% increase in non-glycolytic acidification/20K for dogs treated with Metformin drug compared to dogs in control group or those treated with another drug
- We expect a 40.93% increase in non-glycolytic acidification/20K for dogs treated with Rapamycin drug compared to dogs in control group or those treated with another drug
- We expect a 38.57% increase in non-glycolytic acidification/20K for dogs treated with Resveratrol drug compared to dogs in control group or those treated with another drug

3 Glycolysis/20K

3.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	2.6498	0.4111	140.7656	6.4453	<0.0001
Age.ClassOld	0.0433	0.2794	247.4918	0.1549	0.9286
SexM	-0.0296	0.1207	125.1348	-0.2449	0.9286
Size.ClassL	-0.0624	0.1725	247.5457	-0.3618	0.9286
breed.lifespan	0.1002	0.0341	131.6454	2.9397	0.0349
treatmentMetformin	0.4230	0.2157	332.1253	1.9607	0.3045
treatmentRapamycin	0.1152	0.1435	317.2953	0.8027	0.9156
treatmentResveratrol	0.1763	0.1358	311.2920	1.2986	0.8070
Age.ClassOld:treatmentMetformin	0.3318	0.4464	338.9450	0.7433	0.9156
Age.ClassOld:treatmentRapamycin	0.1385	0.3000	323.5504	0.4617	0.9286
Age.ClassOld:treatmentResveratrol	-0.0762	0.2658	311.2920	-0.2866	0.9286
Size.ClassL:treatmentMetformin	-0.1792	0.2395	330.4906	-0.7485	0.9156
Size.ClassL:treatmentRapamycin	-0.1961	0.1708	315.5268	-1.1482	0.8070
Size.ClassL:treatmentResveratrol	-0.0834	0.1648	311.6656	-0.5059	0.9286
Age.ClassOld:Size.ClassL	0.1659	0.3725	254.4045	0.4453	0.9286
Age.ClassOld:Size.ClassL:treatmentMetformin	-0.6647	0.6003	337.3046	-1.1072	0.8070
Age.ClassOld:Size.ClassL:treatmentRapamycin	-0.0669	0.3883	318.6028	-0.1723	0.9286
Age.ClassOld:Size.ClassL:treatmentResveratrol	0.0160	0.3628	311.3692	0.0441	0.9648

Table 17: Summary of the log-transformed full model for Glycolysis/20K.

3.1.1 Post-hoc Analysis

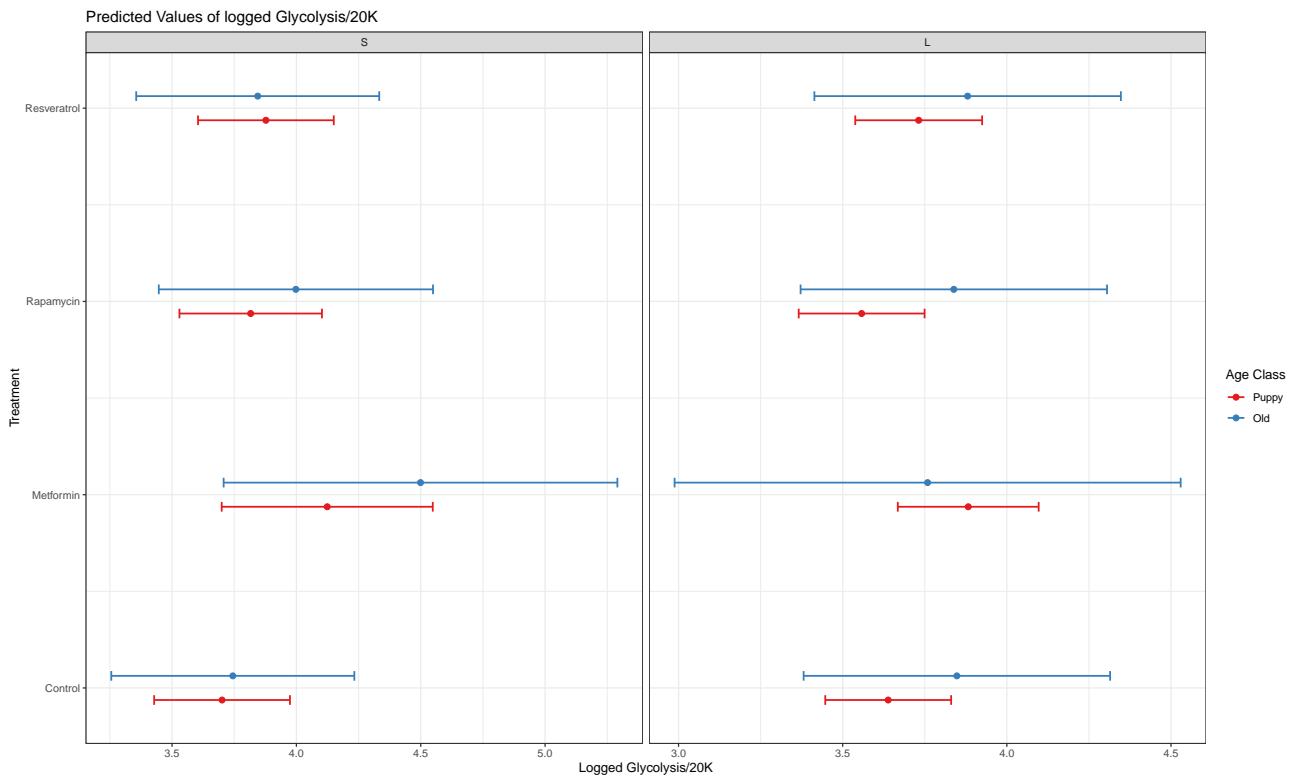


Figure 6: Marginal means for the full log transformed model for Glycolysis/20K.

	Treatment	Age Class	Size Class	Est. Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	3.7010	0.1393	251.7933	3.4267	3.9753
2	Metformin	Puppy	S	4.1240	0.2167	429.7667	3.6981	4.5499
3	Rapamycin	Puppy	S	3.8162	0.1462	282.0323	3.5284	4.1040
4	Resveratrol	Puppy	S	3.8773	0.1393	251.7933	3.6030	4.1516
5	Control	Old	S	3.7443	0.2493	232.7426	3.2531	4.2355
6	Metformin	Old	S	4.4991	0.4043	429.2325	3.7044	5.2938
7	Rapamycin	Old	S	3.9980	0.2814	307.5263	3.4442	4.5518
8	Resveratrol	Old	S	3.8444	0.2493	232.7426	3.3532	4.3357
9	Control	Puppy	L	3.6386	0.0978	241.0571	3.4459	3.8313
10	Metformin	Puppy	L	3.8824	0.1095	315.2875	3.6669	4.0978
11	Rapamycin	Puppy	L	3.5577	0.0978	241.0571	3.3650	3.7504
12	Resveratrol	Puppy	L	3.7315	0.0986	245.8601	3.5373	3.9257
13	Control	Old	L	3.8478	0.2381	245.9432	3.3787	4.3169
14	Metformin	Old	L	3.7587	0.3936	430.9884	2.9850	4.5323
15	Rapamycin	Old	L	3.8385	0.2381	245.9432	3.3694	4.3075
16	Resveratrol	Old	L	3.8805	0.2381	245.9432	3.4115	4.3496

Table 18: Summary of the marginal means for the full log transformed model for Glycolysis/20k.

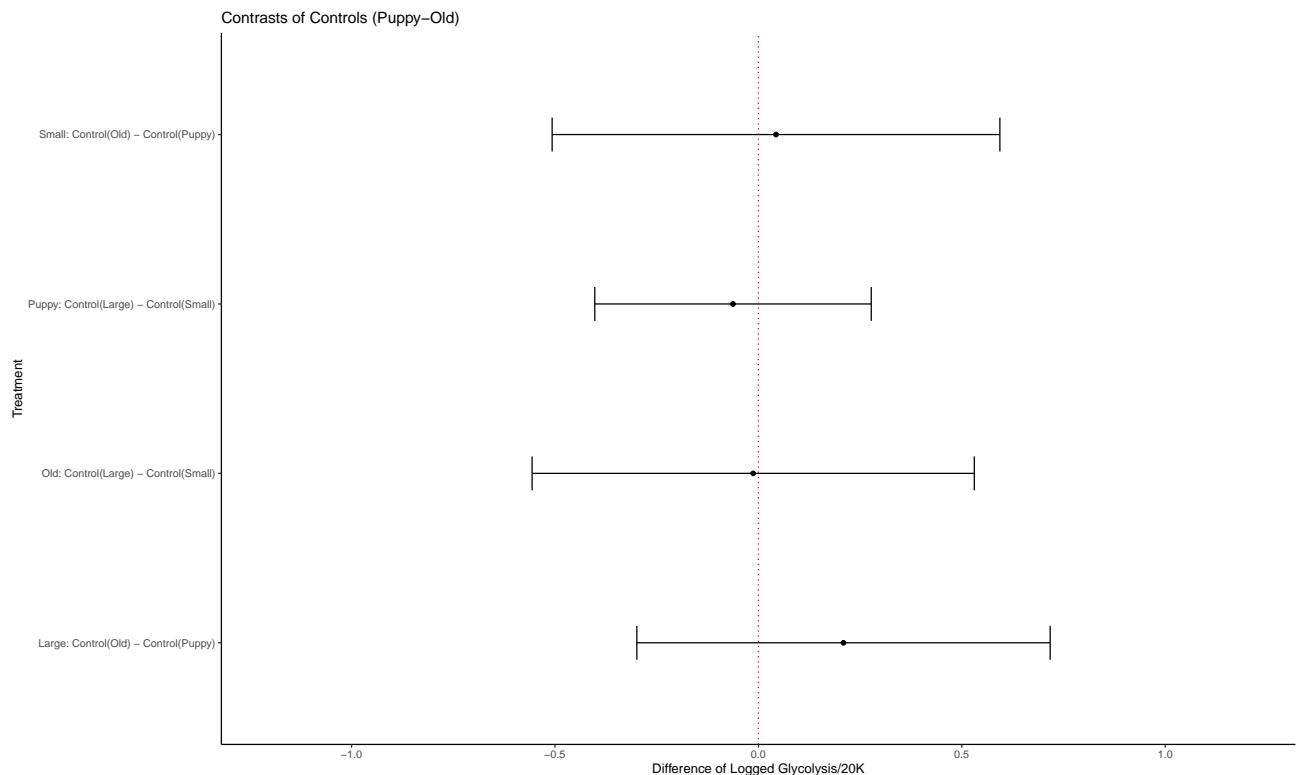


Figure 7: Pairwise treatment contrasts for the full log transformed model for Total Glycolysis/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	0.2092	0.2579	244.7956	0.8111	0.9630	-0.2988	0.7171
2	Small: Control(Old) - Control(Puppy)	0.0433	0.2794	243.8742	0.1549	0.9630	-0.5070	0.5936
3	Puppy: Control(Large) - Control(Small)	-0.0624	0.1725	243.9285	-0.3618	0.9630	-0.4022	0.2774
4	Old: Control(Large) - Control(Small)	-0.0128	0.2758	226.5566	-0.0464	0.9630	-0.5563	0.5307

Table 19: Summary of the pairwise control contrast for the full log transformed model for Glycolysis/20k.

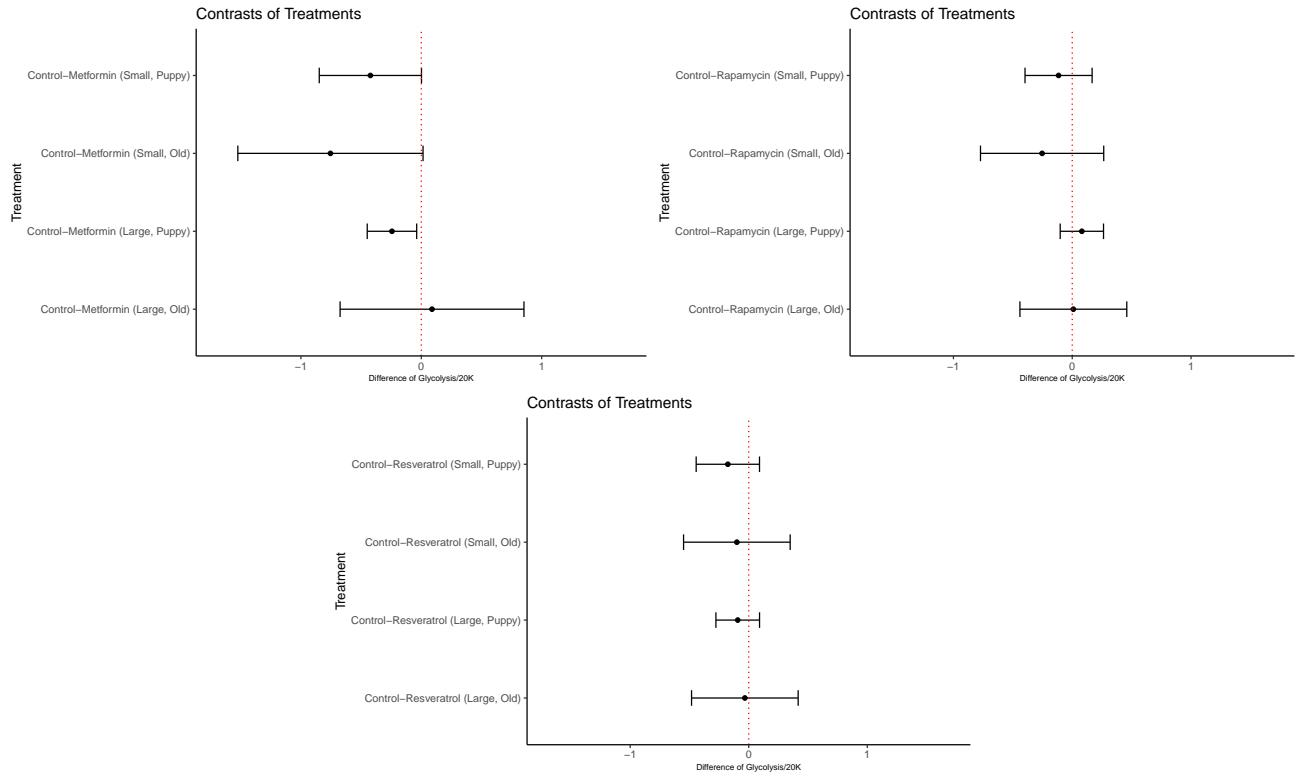


Figure 8: Pairwise treatment contrasts for the full log transformed model for Total Glycolysis/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control-Metformin (Small, Puppy)	-0.4230	0.2159	329.4919	-1.9592	0.2177	-0.8477	0.0017
2	Control-Metformin (Large, Puppy)	-0.2437	0.1044	315.7892	-2.3351	0.2177	-0.4491	-0.0384
3	Control-Metformin (Small, Old)	-0.7548	0.3911	339.0096	-1.9301	0.2177	-1.5240	0.0144
4	Control-Metformin (Large, Old)	0.0891	0.3882	333.2403	0.2296	0.9668	-0.6745	0.8528
5	Control-Rapamycin (Small, Puppy)	-0.1152	0.1435	314.4056	-0.8025	0.6343	-0.3975	0.1672
6	Control-Rapamycin (Large, Puppy)	0.0809	0.0926	308.3087	0.8736	0.6343	-0.1014	0.2632
7	Control-Rapamycin (Small, Old)	-0.2537	0.2636	322.5343	-0.9622	0.6343	-0.7723	0.2650
8	Control-Rapamycin (Large, Old)	0.0093	0.2285	308.3087	0.0409	0.9674	-0.4403	0.4590
9	Control-Resveratrol (Small, Puppy)	-0.1763	0.1358	308.3087	-1.2986	0.5852	-0.4434	0.0908
10	Control-Resveratrol (Large, Puppy)	-0.0929	0.0935	309.4875	-0.9933	0.6343	-0.2769	0.0911
11	Control-Resveratrol (Small, Old)	-0.1001	0.2285	308.3087	-0.4381	0.8821	-0.5498	0.3495
12	Control-Resveratrol (Large, Old)	-0.0327	0.2285	308.3087	-0.1432	0.9668	-0.4824	0.4169

Table 20: Summary of the pairwise treatment contrasts for the full log transformed model for Glycolysis/20K.

3.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	2.5544	0.3321	130.4050	7.6920	<0.0001
SexM	-0.0373	0.1154	129.6396	-0.3231	0.7471
breed.lifespan	0.1143	0.0293	133.4681	3.9022	0.0002

Table 21: Summary of the log-transformed best subsets model for Glycolysis/20K.

3.3 Analysis

A log transform of Glycolysis/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that breed lifespan is significantly associated with glycolysis/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 2.5544 - 0.0373X_{1i} + 0.01143X_{2i} + \epsilon_i.$$

Where

$$Y_i = \text{Glycolysis}/20K$$

α_i = The random intercept for dog i

X_{1i} = Sex (1=Male, 0=Female)

X_{2i} = Breed lifespan (in years)

for each observation $i = 1, 2, \dots, n$.

- We expect a 3.67% decrease in glycolysis/20K for male dogs compared to female dogs
- For every increase in breed lifespan by a year, we expect a 1.15% increase in glycolysis/20K

4 Glycolytic Capacity/20K

4.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	2.7830	0.3321	143.7628	8.3809	<0.0001
Age.ClassOld	0.1993	0.2322	272.6861	0.8584	0.7046
SexM	-0.0691	0.0969	124.5993	-0.7133	0.7155
Size.ClassL	0.0443	0.1434	272.7314	0.3092	0.9089
breed.lifespan	0.0943	0.0274	132.5204	3.4383	0.0070
treatmentMetformin	0.3497	0.1910	336.5135	1.8313	0.2446
treatmentRapamycin	0.2675	0.1274	318.1343	2.1006	0.1641
treatmentResveratrol	0.2778	0.1206	311.1344	2.3029	0.1317
Age.ClassOld:treatmentMetformin	0.0202	0.3947	344.4190	0.0512	0.9592
Age.ClassOld:treatmentRapamycin	0.1235	0.2660	325.5014	0.4642	0.8890
Age.ClassOld:treatmentResveratrol	-0.0938	0.2362	311.1344	-0.3973	0.8890
Size.ClassL:treatmentMetformin	-0.2406	0.2120	334.4966	-1.1347	0.6028
Size.ClassL:treatmentRapamycin	-0.1570	0.1517	316.0688	-1.0352	0.6028
Size.ClassL:treatmentResveratrol	-0.1554	0.1465	311.5672	-1.0609	0.6028
Age.ClassOld:Size.ClassL	0.0570	0.3101	280.5926	0.1837	0.9592
Age.ClassOld:Size.ClassL:treatmentMetformin	-0.5882	0.5310	342.6349	-1.1078	0.6028
Age.ClassOld:Size.ClassL:treatmentRapamycin	-0.2716	0.3447	319.6876	-0.7881	0.7057
Age.ClassOld:Size.ClassL:treatmentResveratrol	-0.0214	0.3224	311.2238	-0.0664	0.9592

Table 22: Summary of the log-transformed full model for Glycolytic Capacity/20K.

4.1.1 Post-hoc Analysis

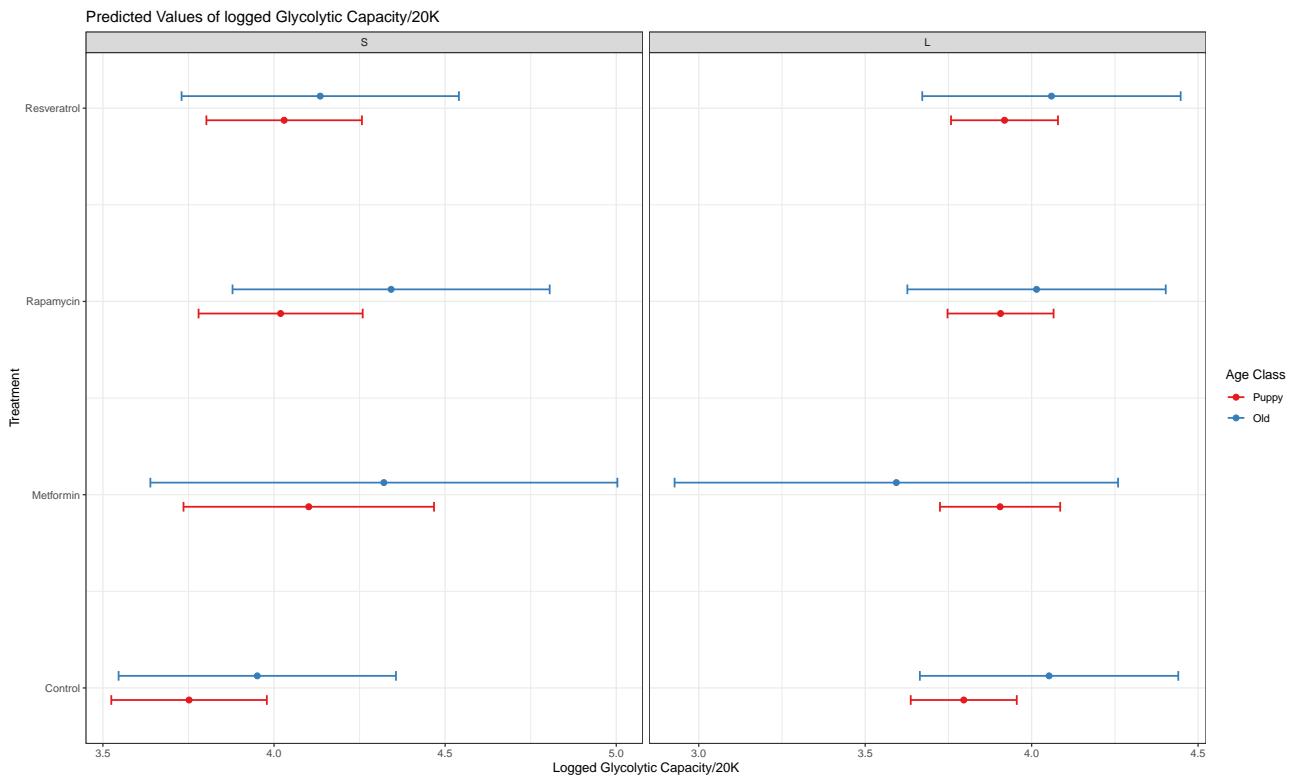


Figure 9: Marginal means for the full log transformed model for Glycolytic Capacity/20K.

	Treatment	Age Class	Size Class	Est.	Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	3.7518	0.1159	278.6785	3.5235	3.9800	
2	Metformin	Puppy	S	4.1015	0.1869	430.9786	3.7341	4.4689	
3	Rapamycin	Puppy	S	4.0193	0.1224	309.9822	3.7785	4.2601	
4	Resveratrol	Puppy	S	4.0296	0.1159	278.6785	3.8014	4.2578	
5	Control	Old	S	3.9511	0.2068	256.6482	3.5439	4.3583	
6	Metformin	Old	S	4.3210	0.3485	430.9279	3.6359	5.0061	
7	Rapamycin	Old	S	4.3421	0.2365	333.7532	3.8768	4.8074	
8	Resveratrol	Old	S	4.1351	0.2068	256.6482	3.7279	4.5422	
9	Control	Puppy	L	3.7961	0.0813	266.2859	3.6361	3.9561	
10	Metformin	Puppy	L	3.9052	0.0922	343.6178	3.7239	4.0865	
11	Rapamycin	Puppy	L	3.9066	0.0813	266.2859	3.7466	4.0666	
12	Resveratrol	Puppy	L	3.9185	0.0820	271.4175	3.7571	4.0799	
13	Control	Old	L	4.0524	0.1980	271.9874	3.6625	4.4422	
14	Metformin	Old	L	3.5935	0.3402	430.0943	2.9248	4.2622	
15	Rapamycin	Old	L	4.0148	0.1980	271.9874	3.6249	4.4046	
16	Resveratrol	Old	L	4.0595	0.1980	271.9874	3.6697	4.4494	

Table 23: Summary of the marginal means for the full log transformed model for Glycolytic Capacity/20k.

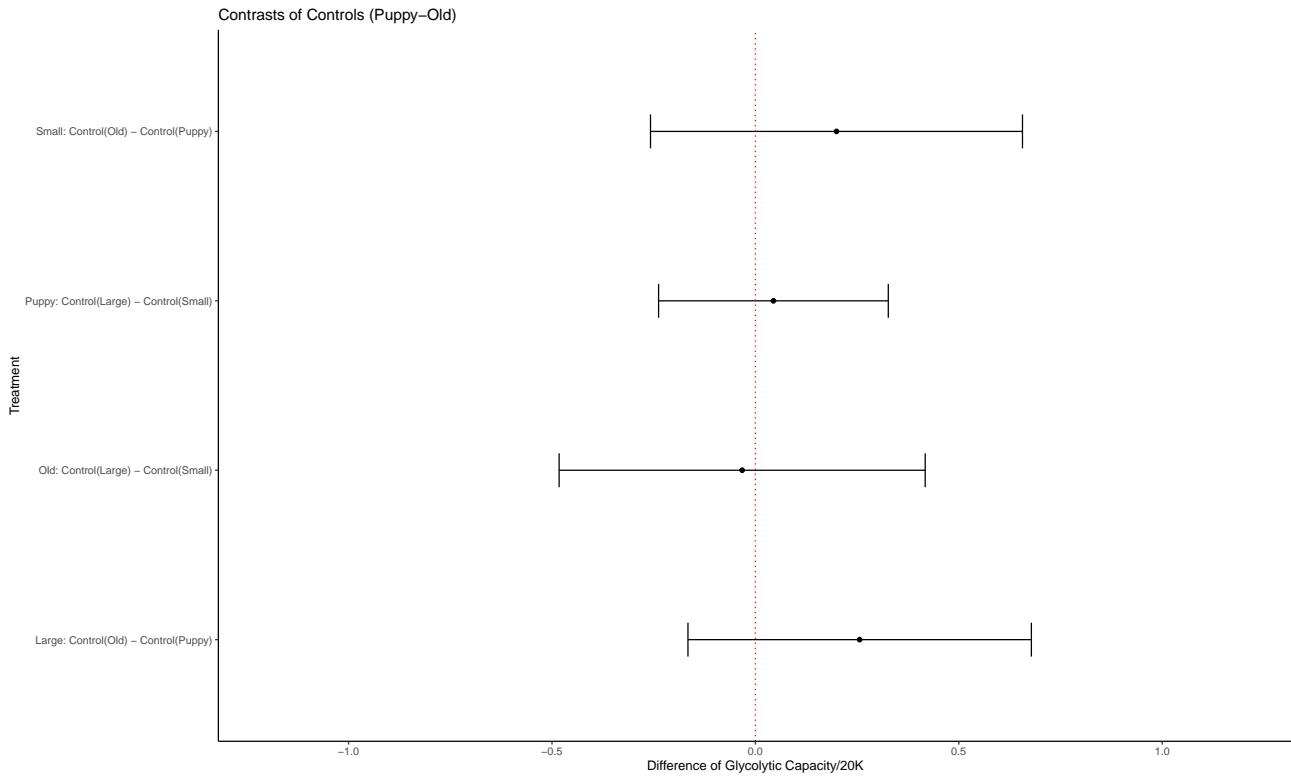


Figure 10: Pairwise treatment contrasts for the full log transformed model for Glycolytic Capacity/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	0.2563	0.2144	270.6471	1.1954	0.7829	-0.1658	0.6783
2	Small: Control(Old) - Control(Puppy)	0.1993	0.2322	269.5915	0.8584	0.7829	-0.2578	0.6565
3	Puppy: Control(Large) - Control(Small)	0.0443	0.1434	269.6371	0.3092	0.8868	-0.2380	0.3266
4	Old: Control(Large) - Control(Small)	-0.0326	0.2284	249.3330	-0.1425	0.8868	-0.4825	0.4173

Table 24: Summary of the pairwise control contrast for the full log transformed model for Glycolytic Capacity/20k.

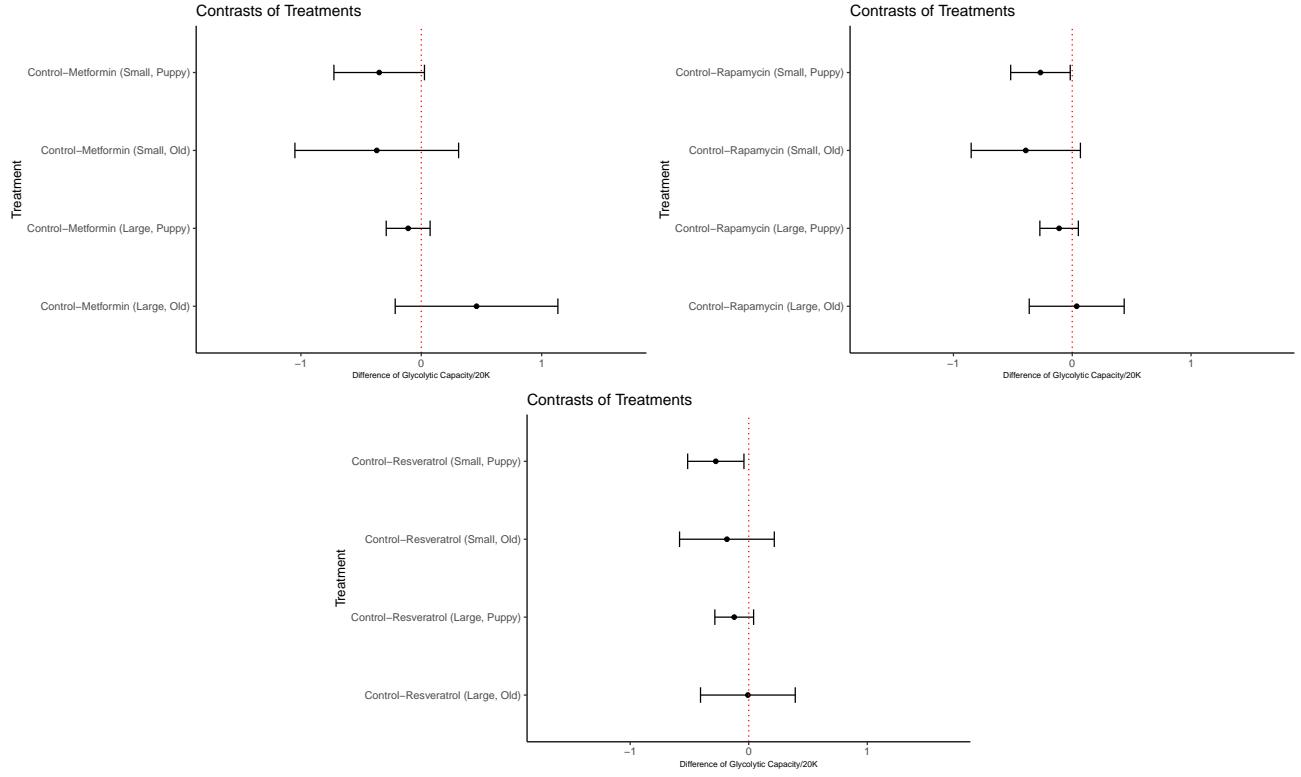


Figure 11: Pairwise treatment contrasts for the full log transformed model for Glycolytic Capacity/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control-Metformin (Small, Puppy)	-0.3497	0.1911	334.2237	-1.8297	0.2728	-0.7257	0.0263
2	Control-Metformin (Large, Puppy)	-0.1091	0.0926	317.4895	-1.1779	0.3596	-0.2914	0.0731
3	Control-Metformin (Small, Old)	-0.3699	0.3457	345.2629	-1.0701	0.3804	-1.0498	0.3100
4	Control-Metformin (Large, Old)	0.4589	0.3435	338.8835	1.3359	0.3128	-0.2168	1.1345
5	Control-Rapamycin (Small, Puppy)	-0.2675	0.1274	315.5520	-2.1001	0.2191	-0.5182	-0.0169
6	Control-Rapamycin (Large, Puppy)	-0.1105	0.0823	308.4537	-1.3426	0.3128	-0.2726	0.0515
7	Control-Rapamycin (Small, Old)	-0.3910	0.2337	325.1247	-1.6730	0.2859	-0.8508	0.0688
8	Control-Rapamycin (Large, Old)	0.0376	0.2031	308.4537	0.1851	0.9308	-0.3620	0.4372
9	Control-Resveratrol (Small, Puppy)	-0.2778	0.1206	308.4537	-2.3029	0.2191	-0.5152	-0.0404
10	Control-Resveratrol (Large, Puppy)	-0.1224	0.0831	309.8175	-1.4733	0.3128	-0.2859	0.0411
11	Control-Resveratrol (Small, Old)	-0.1840	0.2031	308.4537	-0.9060	0.4388	-0.5836	0.2156
12	Control-Resveratrol (Large, Old)	-0.0072	0.2031	308.4537	-0.0353	0.9719	-0.4068	0.3924

Table 25: Summary of the pairwise treatment contrasts for the full log transformed model for Glycolytic Capacity/20K.

4.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	2.7933	0.2663	130.6271	10.4879	<0.0001
SexM	-0.0779	0.0925	129.6452	-0.8419	0.4014
breed.lifespan	0.1103	0.0235	134.2962	4.6871	<0.0001

Table 26: Summary of the log-transformed best subsets model for Glycolytic Capacity/20K.

4.3 Analysis

A log transform of glycolytic capacity/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that breed lifespan is significantly associated with glycolytic capacity/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 2.7933 - 0.0779X_{1i} + 0.1103X_{2i} + \epsilon_i.$$

Where

$$Y_i = \text{Glycolytic capacity/20K}$$

$$\alpha_i = \text{The random intercept for dog } i$$

$$X_{1i} = \text{Sex (1=Male, 0=Female)}$$

$$X_{2i} = \text{Breed lifespan (in years)}$$

for each observation $i = 1, 2, \dots, n$.

- We expect a 7.49% decrease in glycolytic capacity/20K for male dogs compared to female dogs
- For every increase in breed lifespan by an year, we expect a 11.66% increase in glycolytic capacity/20K.

5 Basal/20K

5.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	1.3079	0.4824	134.3137	2.7110	0.0341
Age.ClassOld	0.4753	0.3265	237.0662	1.4556	0.3775
SexM	-0.0265	0.1417	119.1334	-0.1872	0.8519
Size.ClassL	-0.2174	0.2016	237.1214	-1.0783	0.5184
breed.lifespan	0.1285	0.0400	125.6526	3.2100	0.0152
treatmentMetformin	-0.6430	0.2501	324.2400	-2.5707	0.0381
treatmentRapamycin	0.5003	0.1663	309.3782	3.0081	0.0171
treatmentResveratrol	0.1406	0.1573	303.3416	0.8939	0.5581
Age.ClassOld:treatmentMetformin	0.3000	0.5177	331.1584	0.5795	0.6278
Age.ClassOld:treatmentRapamycin	-1.1285	0.3478	315.6767	-3.2445	0.0152
Age.ClassOld:treatmentResveratrol	0.2015	0.3081	303.3416	0.6542	0.6278
Size.ClassL:treatmentMetformin	0.2963	0.2784	322.7892	1.0643	0.5184
Size.ClassL:treatmentRapamycin	0.1059	0.1980	307.5989	0.5352	0.6278
Size.ClassL:treatmentResveratrol	0.2442	0.1911	303.7173	1.2780	0.4550
Age.ClassOld:Size.ClassL	-0.2499	0.4353	243.8710	-0.5740	0.6278
Age.ClassOld:Size.ClassL:treatmentMetformin	-0.6864	0.6965	329.5245	-0.9855	0.5320
Age.ClassOld:Size.ClassL:treatmentRapamycin	0.6889	0.4502	310.6933	1.5304	0.3775
Age.ClassOld:Size.ClassL:treatmentResveratrol	-0.2983	0.4205	303.4192	-0.7094	0.6278

Table 27: Summary of the log-transformed full model for Basal OCR/20K.

5.1.1 Post-hoc Analysis

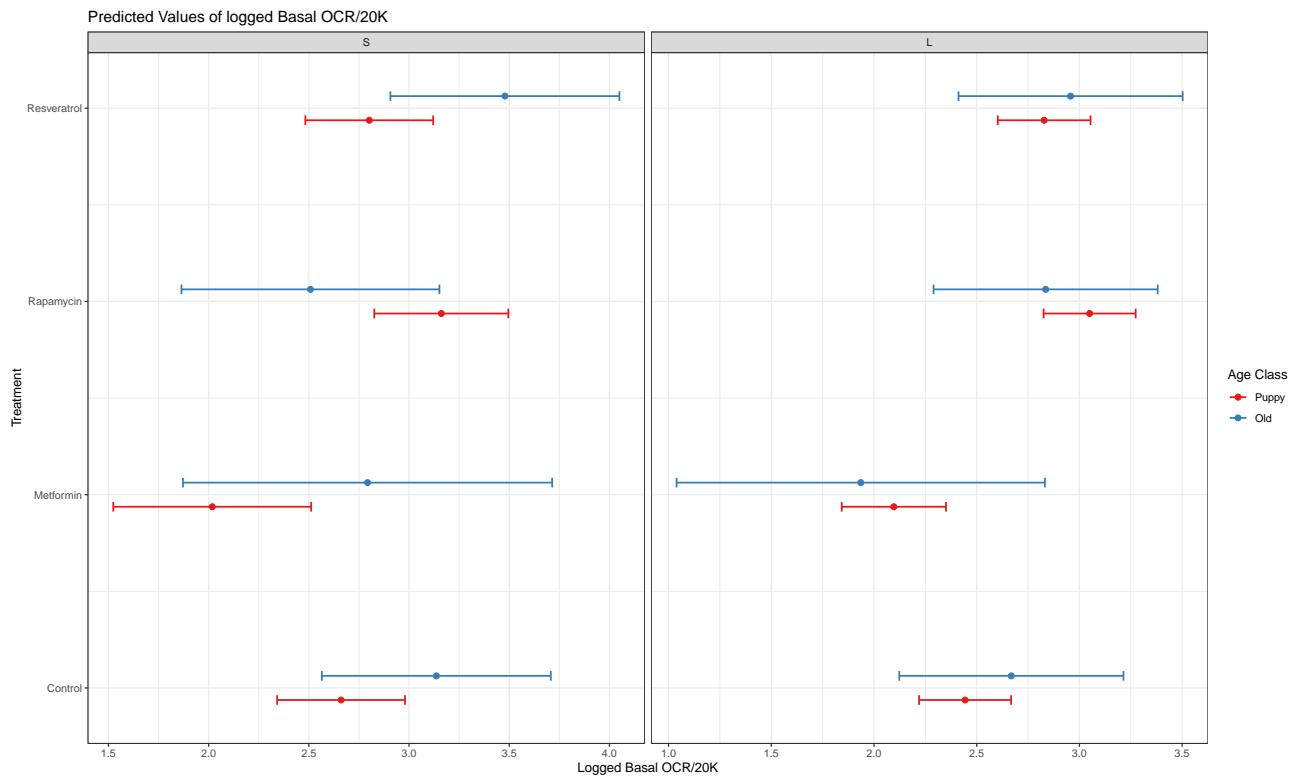


Figure 12: Marginal means for the full log transformed model for Basal OCR/20K.

	Treatment	Age Class	Size Class	Est. Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	2.6609	0.1628	248.3152	2.3403	2.9814
2	Metformin	Puppy	S	2.0178	0.2521	427.4881	1.5223	2.5133
3	Rapamycin	Puppy	S	3.1611	0.1708	278.1880	2.8250	3.4972
4	Resveratrol	Puppy	S	2.8015	0.1628	248.3152	2.4809	3.1221
5	Control	Old	S	3.1361	0.2915	229.7309	2.5617	3.7105
6	Metformin	Old	S	2.7931	0.4705	426.9742	1.8684	3.7178
7	Rapamycin	Old	S	2.5079	0.3285	303.6982	1.8614	3.1544
8	Resveratrol	Old	S	3.4783	0.2915	229.7309	2.9039	4.0527
9	Control	Puppy	L	2.4435	0.1143	237.8946	2.2182	2.6687
10	Metformin	Puppy	L	2.0967	0.1295	319.4367	1.8420	2.3514
11	Rapamycin	Puppy	L	3.0497	0.1143	237.8946	2.8244	3.2749
12	Resveratrol	Puppy	L	2.8283	0.1152	242.6331	2.6013	3.0552
13	Control	Old	L	2.6688	0.2783	242.6181	2.1206	3.2171
14	Metformin	Old	L	1.9356	0.4578	428.9579	1.0358	2.8355
15	Rapamycin	Old	L	2.8355	0.2783	242.6181	2.2872	3.3838
16	Resveratrol	Old	L	2.9569	0.2783	242.6181	2.4087	3.5052

Table 28: Summary of the marginal means for the full log transformed model for Basal OCR/20k.

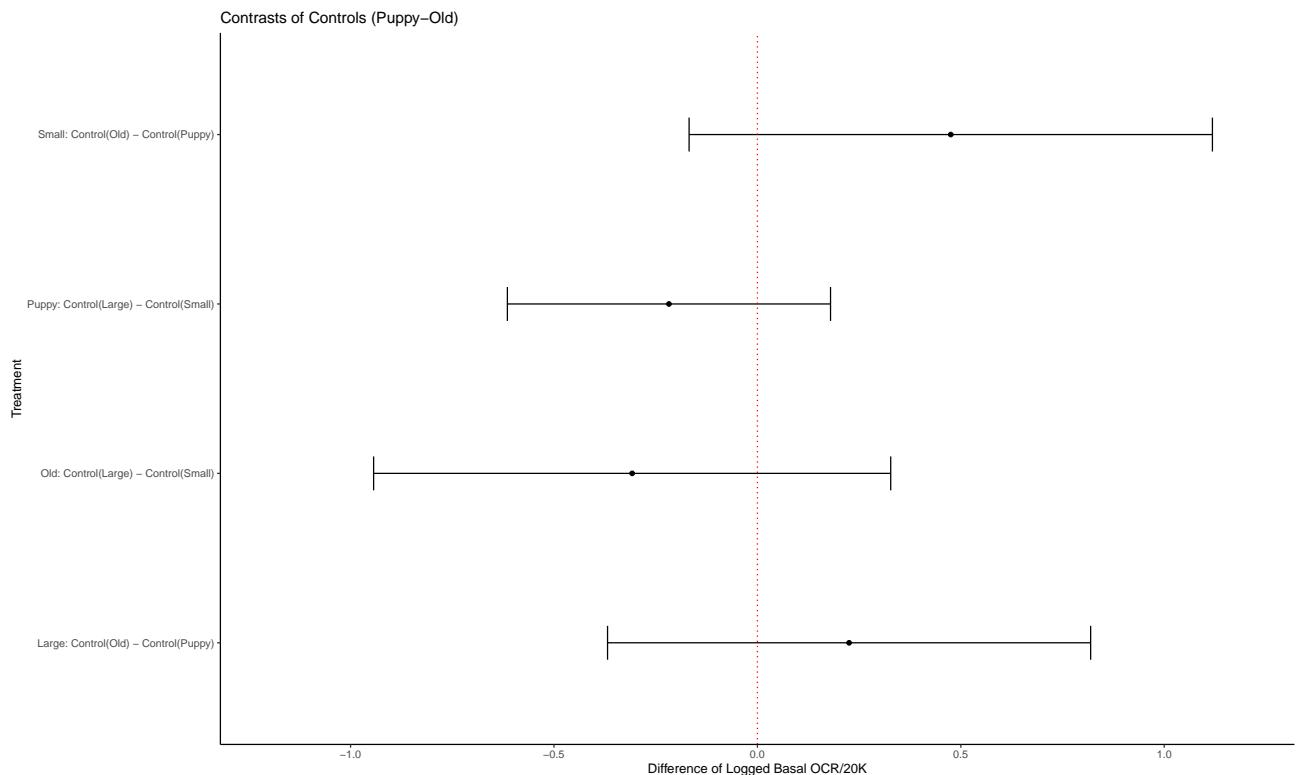


Figure 13: Pairwise treatment contrasts for the full log transformed model for Basal OCR/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	0.2254	0.3014	241.5255	0.7478	0.4553	-0.3683	0.8191
2	Small: Control(Old) - Control(Puppy)	0.4753	0.3265	240.6082	1.4556	0.4544	-0.1679	1.1185
3	Puppy: Control(Large) - Control(Small)	-0.2174	0.2016	240.6632	-1.0783	0.4544	-0.6146	0.1797
4	Old: Control(Large) - Control(Small)	-0.3078	0.3225	223.7352	-0.9546	0.4544	-0.9434	0.3277

Table 29: Summary of the pairwise control contrast for the full log transformed model for Basal OCR/20k.

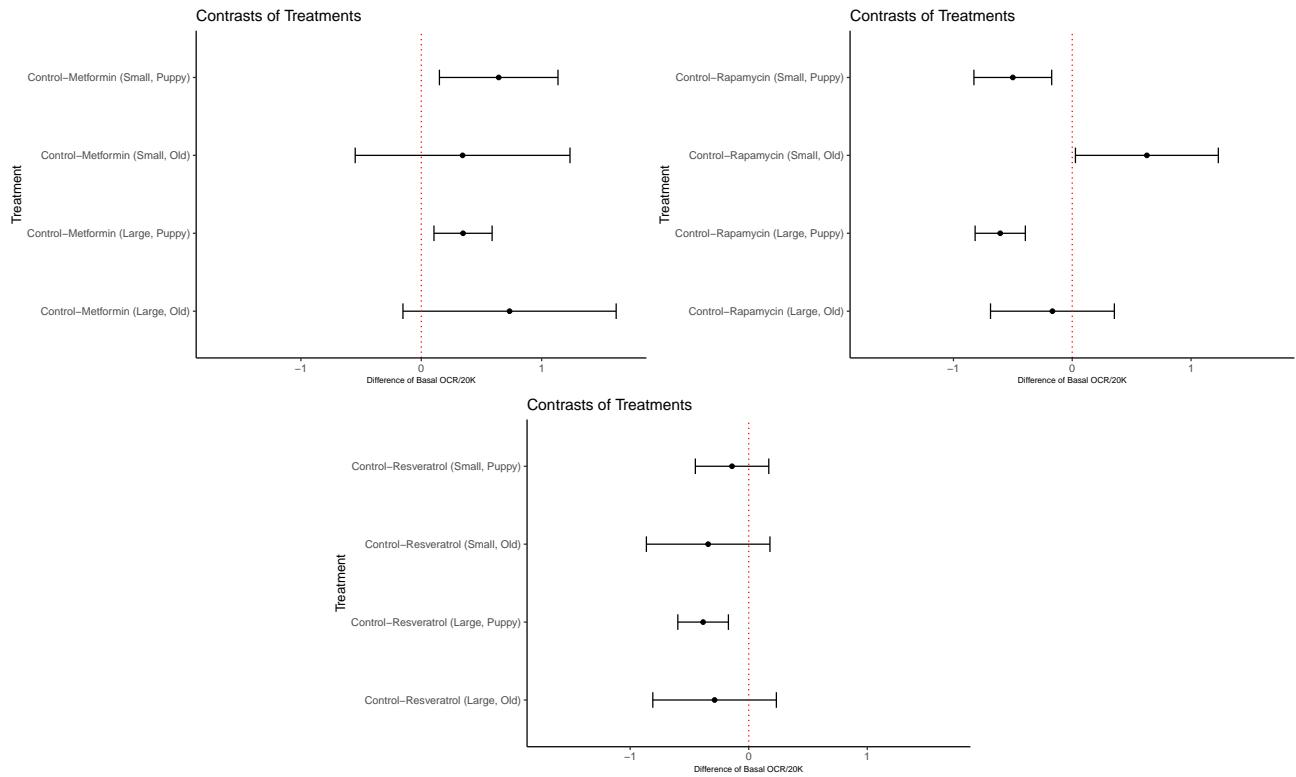


Figure 14: Pairwise treatment contrasts for the full log transformed model for Basal OCR/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control-Metformin (Small, Puppy)	0.6430	0.2503	326.8663	2.5688	0.0256	0.1506	1.1355
2	Control-Metformin (Large, Puppy)	0.3468	0.1227	314.2229	2.8252	0.0151	0.1053	0.5882
3	Control-Metformin (Small, Old)	0.3431	0.4535	336.1740	0.7564	0.4908	-0.5490	1.2352
4	Control-Metformin (Large, Old)	0.7332	0.4502	330.5101	1.6287	0.1788	-0.1523	1.6187
5	Control-Rapamycin (Small, Puppy)	-0.5003	0.1663	312.2430	-3.0075	0.0114	-0.8275	-0.1730
6	Control-Rapamycin (Large, Puppy)	-0.6062	0.1074	306.2936	-5.6452	<0.0001	-0.8175	-0.3949
7	Control-Rapamycin (Small, Old)	0.6282	0.3057	320.1701	2.0553	0.0813	0.0269	1.2296
8	Control-Rapamycin (Large, Old)	-0.1667	0.2649	306.2936	-0.6293	0.5296	-0.6878	0.3545
9	Control-Resveratrol (Small, Puppy)	-0.1406	0.1573	306.2936	-0.8939	0.4465	-0.4503	0.1690
10	Control-Resveratrol (Large, Puppy)	-0.3848	0.1084	307.4449	-3.5502	0.0027	-0.5981	-0.1715
11	Control-Resveratrol (Small, Old)	-0.3422	0.2649	306.2936	-1.2920	0.2960	-0.8634	0.1790
12	Control-Resveratrol (Large, Old)	-0.2881	0.2649	306.2936	-1.0878	0.3701	-0.8093	0.2331

Table 30: Summary of the pairwise treatment contrasts for the full log transformed model for Basal OCR/20K.

5.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	0.9923	0.3981	130.1510	2.4925	0.0167
SexM	-0.0764	0.1365	122.5997	-0.5603	0.5763
breed.lifespan	0.1532	0.0348	127.8212	4.4078	0.0001
treatmentMetformin	-0.4461	0.1050	324.7036	-4.2493	0.0001
treatmentRapamycin	0.4502	0.0839	314.3941	5.3668	<0.0001
treatmentResveratrol	0.3089	0.0823	312.4852	3.7542	0.0003

Table 31: Summary of the log-transformed best subsets model for Basal OCR/20K.

5.2.1 Post-hoc Analysis

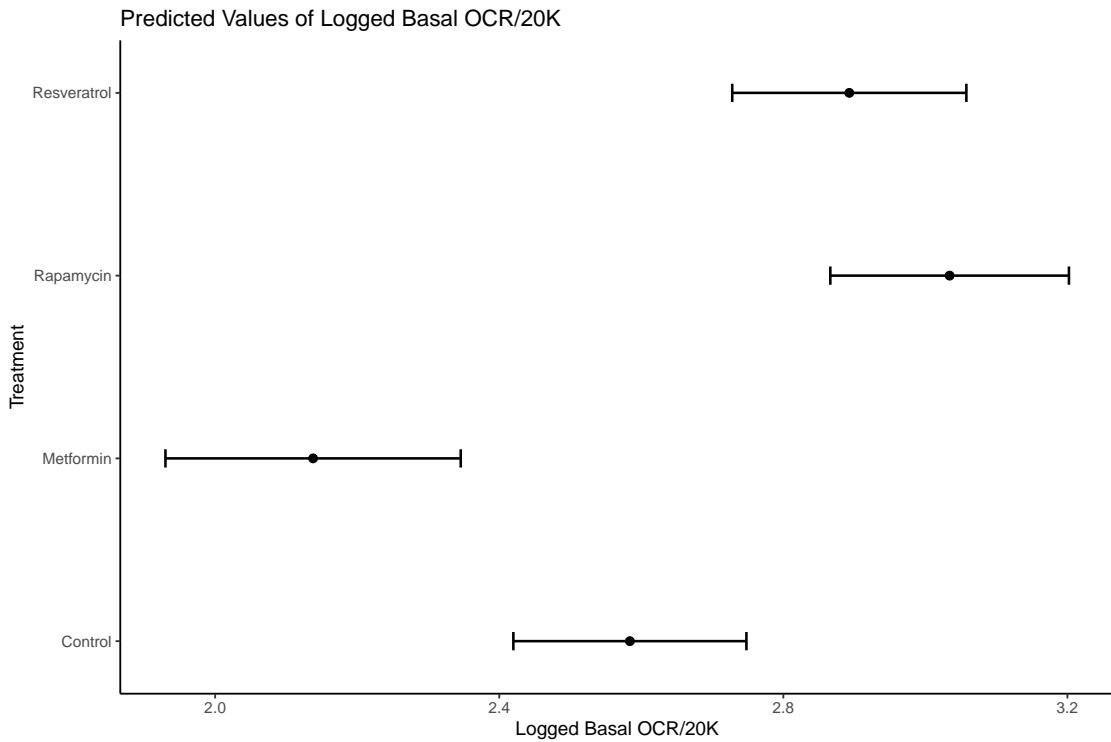


Figure 15: Marginal means for the log-transformed best subsets model for Basal OCR/20K.

	Treatment	Est. Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	2.5839	0.0837	258.2648	2.4191	2.7488
2	Metformin	2.1379	0.1061	397.8471	1.9293	2.3465
3	Rapamycin	3.0341	0.0857	273.6409	2.8654	3.2029
4	Resveratrol	2.8929	0.0841	261.2267	2.7273	3.0585

Table 32: Summary of the marginal means for the log-transformed best subsets model for Basal OCR/20K.

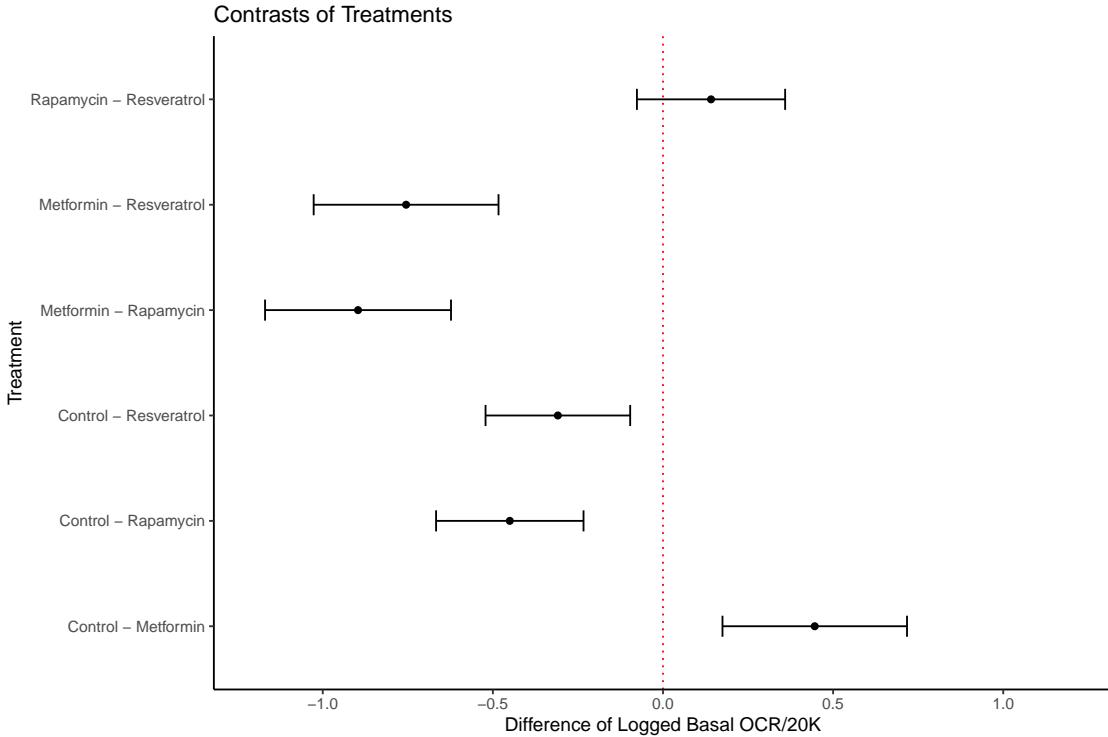


Figure 16: Pairwise treatment contrasts for the log-transformed best subsets model for Basal OCR/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control - Metformin	0.4461	0.1050	328.0063	4.2474	0.0002	0.1749	0.7172
2	Control - Rapamycin	-0.4502	0.0839	317.8786	-5.3664	<0.0001	-0.6669	-0.2335
3	Control - Resveratrol	-0.3089	0.0823	316.0014	-3.7541	0.0012	-0.5215	-0.0964
4	Metformin - Rapamycin	-0.8963	0.1058	328.0011	-8.4693	<0.0001	-1.1695	-0.6230
5	Metformin - Resveratrol	-0.7550	0.1052	327.4188	-7.1769	<0.0001	-1.0267	-0.4833
6	Rapamycin - Resveratrol	0.1412	0.0843	318.5610	1.6749	0.3387	-0.0766	0.3591

Table 33: Summary of the pairwise treatment contrasts for the log-transformed best subsets model for Basal OCR/20K.

5.3 Analysis

A log transform of Basal OCR/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that breed lifespan and treatment with all three drugs (Metformin, Rapamycin and Resveratrol) are significantly associated with Basal

OCR/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 0.9923 - 0.0764X_{1i} + 0.1532X_{2i} - 0.4461X_{3i} + 0.4502X_{4i} + 0.3089X_{5i} + \epsilon_i.$$

Where

$$Y_i = \text{Basal OCR/20K}$$

α_i = The random intercept for dog i

X_{1i} = Sex (1=Male, 0=Female)

X_{2i} = breed lifespan (in years)

X_{3i} = treatment group (1= Metformin, 0= Other)

X_{4i} = treatment group (1= Rapamycin, 0= Other)

X_{5i} = treatment group (1= Resveratrol, 0= Other)

for each observation $i = 1, 2, \dots, n$.

- We expect a 7.36% decrease in Basal OCR/20K for male dogs compared to female dogs
- For every increase in breed lifespan by an year, we expect a 16.56% increase in Basal OCR/20K
- We expect a 35.99% decrease in Basal OCR/20K for dogs treated with Metformin drug compared to dogs in control group or those treated with another drug
- We expect a 56.86% increase in Basal OCR/20K for dogs treated with Rapamycin drug compared to dogs in control group or those treated with another drug
- We expect a 36.19% increase in Basal OCR/20K for dogs treated with Resveratrol drug compared to dogs in control group or those treated with another drug

6 Proton Leak/20K

6.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	1.5933	0.4378	129.7426	3.6395	0.0035
Age.ClassOld	0.3057	0.2858	205.1333	1.0694	0.5151
SexM	-0.2919	0.1296	118.7252	-2.2521	0.1569
Size.ClassL	-0.2637	0.1765	205.1830	-1.4939	0.3923
breed.lifespan	0.0595	0.0365	123.4664	1.6305	0.3799
treatmentMetformin	-0.1909	0.1970	317.0677	-0.9687	0.5456
treatmentRapamycin	0.4926	0.1306	306.2889	3.7721	0.0035
treatmentResveratrol	0.1042	0.1234	301.5732	0.8441	0.5528
Age.ClassOld:treatmentMetformin	0.8136	0.4084	322.4839	1.9922	0.2124
Age.ClassOld:treatmentRapamycin	-0.3878	0.2735	311.1519	-1.4179	0.3923
Age.ClassOld:treatmentResveratrol	0.2749	0.2416	301.5732	1.1380	0.5121
Size.ClassL:treatmentMetformin	0.0526	0.2192	316.0375	0.2398	0.9133
Size.ClassL:treatmentRapamycin	-0.0141	0.1554	304.9018	-0.0907	0.9371
Size.ClassL:treatmentResveratrol	0.0358	0.1501	302.0194	0.2383	0.9133
Age.ClassOld:Size.ClassL	-0.0301	0.3804	210.3328	-0.0790	0.9371
Age.ClassOld:Size.ClassL:treatmentMetformin	-0.7477	0.5492	321.1200	-1.3614	0.3923
Age.ClassOld:Size.ClassL:treatmentRapamycin	0.2329	0.3536	307.2950	0.6586	0.6565
Age.ClassOld:Size.ClassL:treatmentResveratrol	-0.2868	0.3299	301.6656	-0.8696	0.5528

Table 34: Summary of the log-transformed full model for Proton Leak/20K.

6.1.1 Post-hoc Analysis

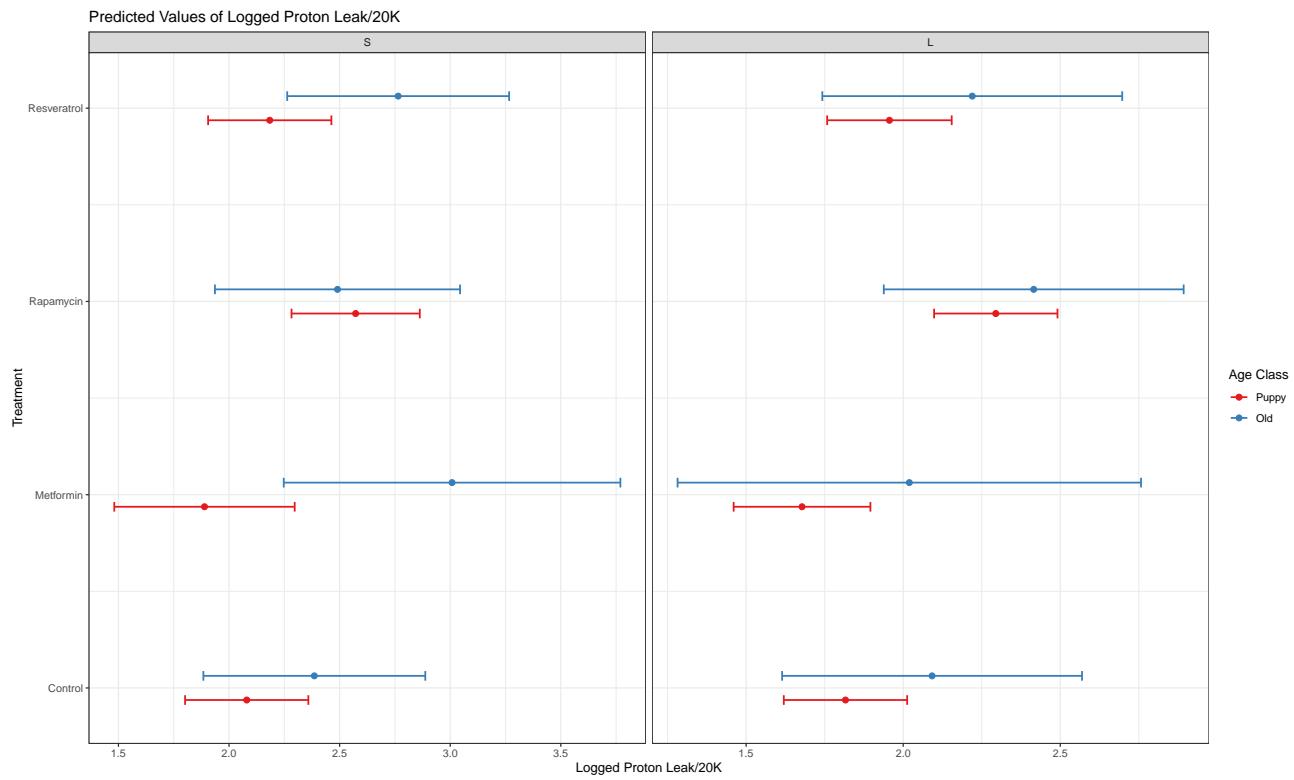


Figure 17: Marginal means for the full log transformed model for Proton Leak/20K.

	Treatment	Age Class	Size Class	Est. Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	2.0799	0.1422	215.4300	1.7997	2.3602
2	Metformin	Puppy	S	1.8891	0.2082	415.3767	1.4799	2.2983
3	Rapamycin	Puppy	S	2.5725	0.1480	241.0069	2.2811	2.8640
4	Resveratrol	Puppy	S	2.1841	0.1422	215.4300	1.9038	2.4644
5	Control	Old	S	2.3856	0.2560	201.2557	1.8809	2.8903
6	Metformin	Old	S	3.0083	0.3885	415.7986	2.2446	3.7721
7	Rapamycin	Old	S	2.4904	0.2828	265.2572	1.9336	3.0472
8	Resveratrol	Old	S	2.7647	0.2560	201.2557	2.2600	3.2694
9	Control	Puppy	L	1.8163	0.1002	207.4003	1.6188	2.0138
10	Metformin	Puppy	L	1.6780	0.1111	277.2895	1.4593	1.8967
11	Rapamycin	Puppy	L	2.2948	0.1002	207.4003	2.0972	2.4923
12	Resveratrol	Puppy	L	1.9562	0.1011	213.2990	1.7568	2.1556
13	Control	Old	L	2.0919	0.2435	211.0277	1.6118	2.5719
14	Metformin	Old	L	2.0194	0.3766	422.6340	1.2793	2.7596
15	Rapamycin	Old	L	2.4155	0.2435	211.0277	1.9354	2.8955
16	Resveratrol	Old	L	2.2199	0.2435	211.0277	1.7398	2.7000

Table 35: Summary of the marginal means for the full log transformed model for Proton Leak/20k.

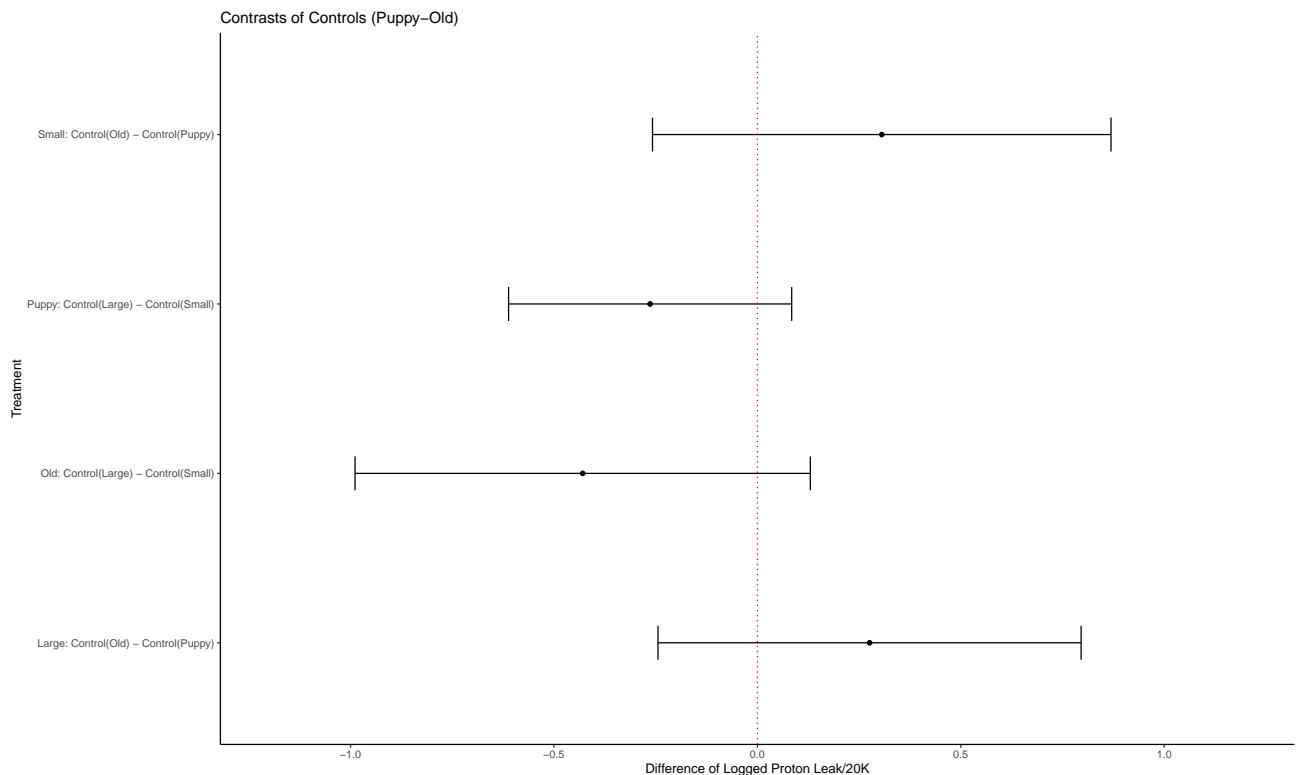


Figure 18: Pairwise treatment contrasts for the full log transformed model for Proton Leak/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	0.2756	0.2638	210.1861	1.0448	0.2973	-0.2444	0.7957
2	Small: Control(Old) - Control(Puppy)	0.3057	0.2858	209.4882	1.0694	0.2973	-0.2578	0.8692
3	Puppy: Control(Large) - Control(Small)	-0.2637	0.1765	209.5380	-1.4939	0.2734	-0.6116	0.0843
4	Old: Control(Large) - Control(Small)	-0.4294	0.2837	196.8852	-1.5135	0.2734	-0.9890	0.1301

Table 36: Summary of the pairwise control contrast for the full log transformed model for Proton Leak/20k.

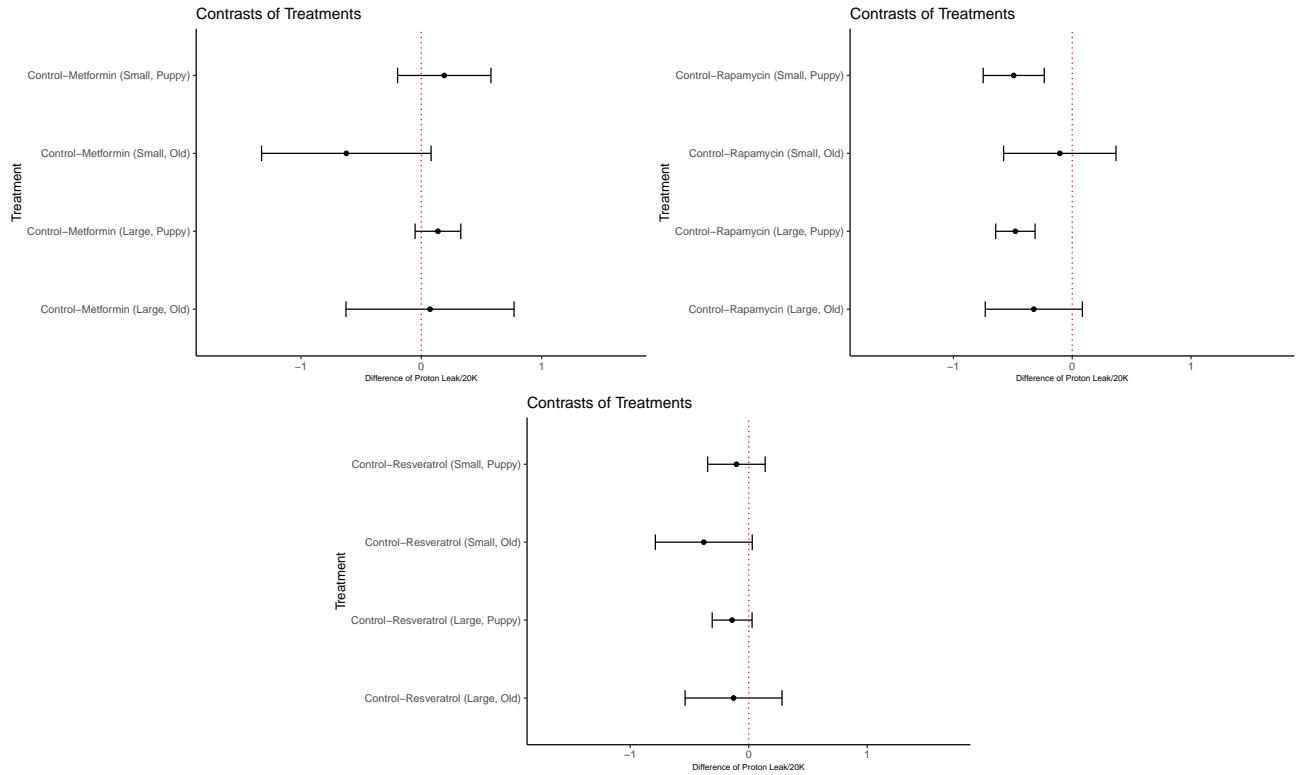


Figure 19: Pairwise treatment contrasts for the full log transformed model for Proton Leak/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control-Metformin (Small, Puppy)	0.1909	0.1971	320.3828	0.9681	0.5006	-0.1970	0.5787
2	Control-Metformin (Large, Puppy)	0.1383	0.0964	311.2606	1.4339	0.2616	-0.0515	0.3280
3	Control-Metformin (Small, Old)	-0.6227	0.3579	327.6341	-1.7400	0.2406	-1.3268	0.0813
4	Control-Metformin (Large, Old)	0.0724	0.3548	322.9979	0.2042	0.8383	-0.6255	0.7704
5	Control-Rapamycin (Small, Puppy)	-0.4926	0.1306	309.8061	-3.7715	0.0012	-0.7496	-0.2356
6	Control-Rapamycin (Large, Puppy)	-0.4785	0.0842	305.1720	-5.6821	<0.0001	-0.6442	-0.3128
7	Control-Rapamycin (Small, Old)	-0.1048	0.2404	315.8988	-0.4359	0.7235	-0.5778	0.3682
8	Control-Rapamycin (Large, Old)	-0.3236	0.2077	305.1720	-1.5579	0.2406	-0.7323	0.0851
9	Control-Resveratrol (Small, Puppy)	-0.1042	0.1234	305.1720	-0.8441	0.5323	-0.3470	0.1386
10	Control-Resveratrol (Large, Puppy)	-0.1399	0.0855	306.5261	-1.6375	0.2406	-0.3081	0.0282
11	Control-Resveratrol (Small, Old)	-0.3791	0.2077	305.1720	-1.8251	0.2406	-0.7878	0.0296
12	Control-Resveratrol (Large, Old)	-0.1280	0.2077	305.1720	-0.6163	0.6458	-0.5367	0.2807

Table 37: Summary of the pairwise treatment contrasts for the full log transformed model for Proton Leak/20K.

6.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	2.3850	0.1266	149.4744	18.8394	<0.0001
SexM	-0.3064	0.1308	122.0570	-2.3418	0.0250
Size.ClassL	-0.3974	0.1357	124.1541	-2.9290	0.0081
treatmentMetformin	-0.1219	0.0811	320.5837	-1.5024	0.1340
treatmentRapamycin	0.4441	0.0647	312.4612	6.8639	<0.0001
treatmentResveratrol	0.1522	0.0636	311.3220	2.3924	0.0250

Table 38: Summary of the log-transformed best subsets model for Proton Leak/20K.

6.2.1 Post-hoc Analysis

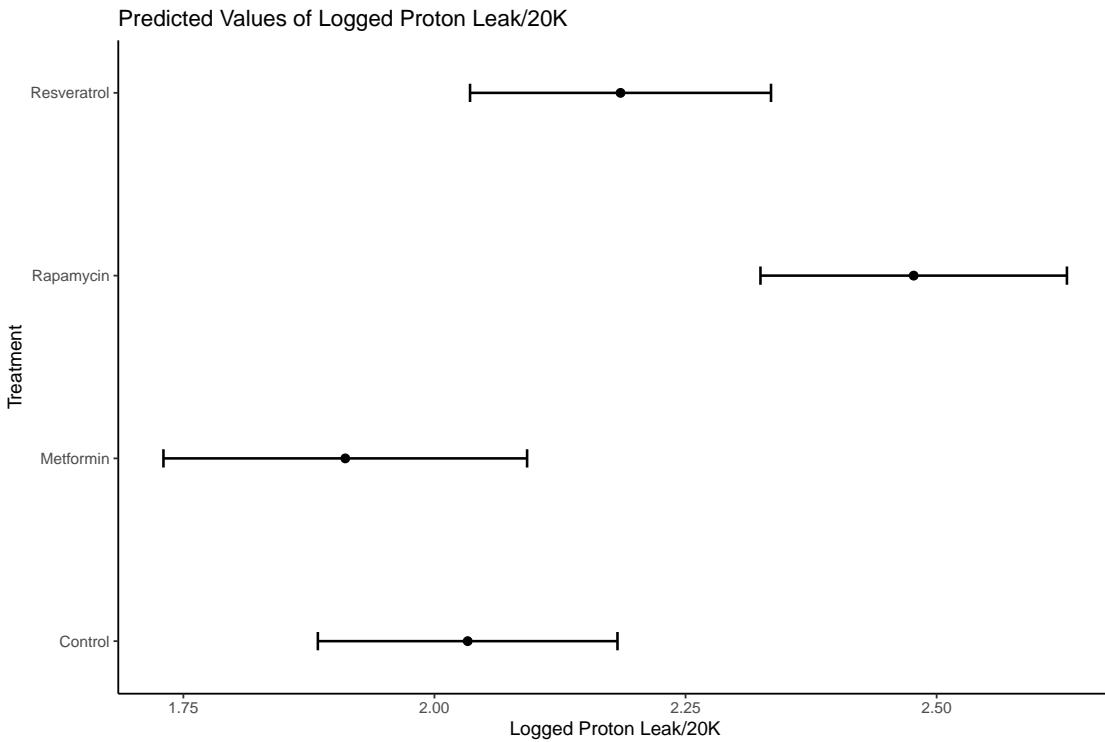


Figure 20: Marginal means for the log-transformed best subsets model for Proton Leak/20K.

	Treatment	Est. Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	2.0331	0.0761	210.0708	1.8831	2.1831
2	Metformin	1.9112	0.0924	342.1795	1.7295	2.0929
3	Rapamycin	2.4772	0.0778	224.8542	2.3238	2.6306
4	Resveratrol	2.1853	0.0764	212.9429	2.0346	2.3360

Table 39: Summary of the marginal means for the log-transformed best subsets model for Proton Leak/20K.

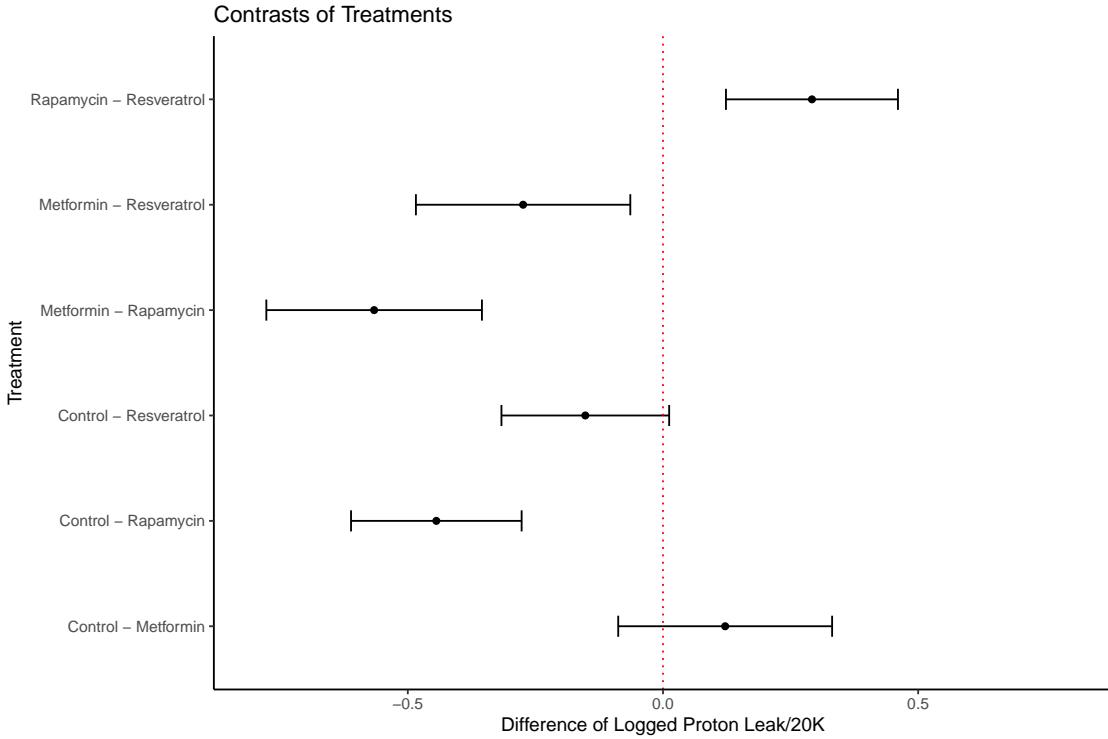


Figure 21: Pairwise treatment contrasts for the log-transformed best subsets model for Proton Leak/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control - Metformin	0.1219	0.0811	323.9973	1.5019	0.4375	-0.0877	0.3314
2	Control - Rapamycin	-0.4441	0.0647	316.0172	-6.8634	<0.0001	-0.6112	-0.2770
3	Control - Resveratrol	-0.1522	0.0636	314.8971	-2.3924	0.0806	-0.3165	0.0121
4	Metformin - Rapamycin	-0.5659	0.0818	323.6239	-6.9195	<0.0001	-0.7772	-0.3547
5	Metformin - Resveratrol	-0.2741	0.0813	323.3522	-3.3695	0.0047	-0.4841	-0.0640
6	Rapamycin - Resveratrol	0.2919	0.0652	316.7410	4.4742	0.0001	0.1234	0.4604

Table 40: Summary of the pairwise treatment contrasts for the log-transformed best subsets model for Proton Leak/20K.

6.3 Analysis

A log transform of Proton leak/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that sex, size class and treatment with drugs Rapamycin and Resveratrol are significantly associated with Proton Leak/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 2.3850 - 0.3064X_{1i} - 0.3974X_{2i} - 0.1219X_{3i} + 0.4441X_{4i} + 0.1522X_{5i} + \epsilon_i.$$

Where

$$Y_i = \text{Proton Leak}/20K$$

α_i = The random intercept for dog i

X_{1i} = Sex (1=Male, 0=Female)

X_{2i} = Size class (1= Large, 0= Small)

X_{3i} = treatment group (1= Metformin, 0= Other)

X_{4i} = treatment group (1= Rapamycin, 0= Other)

X_{5i} = treatment group (1= Resveratrol, 0= Other)

for each observation $i = 1, 2, \dots, n$.

- We expect a 26.39% decrease in Proton Leak/20K for male dogs compared to female dogs
- We expect a 32.79% decrease in Proton Leak/20K for large dogs compared to small dogs
- We expect a 11.48% decrease in Proton Leak/20K for dogs treated with Metformin drug compared to dogs in control group or those treated with another drug
- We expect a 55.91% increase in Proton Leak/20K for dogs treated with Rapamycin drug compared to dogs in control group or those treated with another drug
- We expect a 16.44% increase in Proton Leak/20K for dogs treated with Resveratrol drug compared to dogs in control group or those treated with another drug

7 Maximal Respiration/20K

7.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	1.9461	0.4769	136.9460	4.0807	0.0007
Age.ClassOld	0.7110	0.3437	289.0628	2.0688	0.1420
SexM	-0.1610	0.1380	114.0604	-1.1672	0.5307
Size.ClassL	-0.0776	0.2122	289.0862	-0.3658	0.8041
breed.lifespan	0.1230	0.0392	123.7344	3.1365	0.0128
treatmentMetformin	-0.3996	0.2989	331.2309	-1.3368	0.4685
treatmentRapamycin	0.3846	0.2000	307.3676	1.9230	0.1662
treatmentResveratrol	0.0951	0.1897	298.9534	0.5011	0.7928
Age.ClassOld:treatmentMetformin	-0.2295	0.6169	340.8225	-0.3720	0.8041
Age.ClassOld:treatmentRapamycin	-1.9041	0.4172	316.3762	-4.5638	0.0001
Age.ClassOld:treatmentResveratrol	0.0629	0.3714	298.9534	0.1695	0.8887
Size.ClassL:treatmentMetformin	0.1938	0.3329	328.9123	0.5822	0.7928
Size.ClassL:treatmentRapamycin	0.1315	0.2382	304.8762	0.5520	0.7928
Size.ClassL:treatmentResveratrol	0.2417	0.2303	299.4689	1.0494	0.5307
Age.ClassOld:Size.ClassL	-0.4880	0.4595	298.0255	-1.0621	0.5307
Age.ClassOld:Size.ClassL:treatmentMetformin	-0.1163	0.8305	338.9002	-0.1400	0.8887
Age.ClassOld:Size.ClassL:treatmentRapamycin	1.4304	0.5411	309.2856	2.6434	0.0388
Age.ClassOld:Size.ClassL:treatmentResveratrol	-0.2583	0.5069	299.0598	-0.5095	0.7928

Table 41: Summary of the log-transformed full model for Maximal respiration/20K.

7.1.1 Post-hoc Analysis

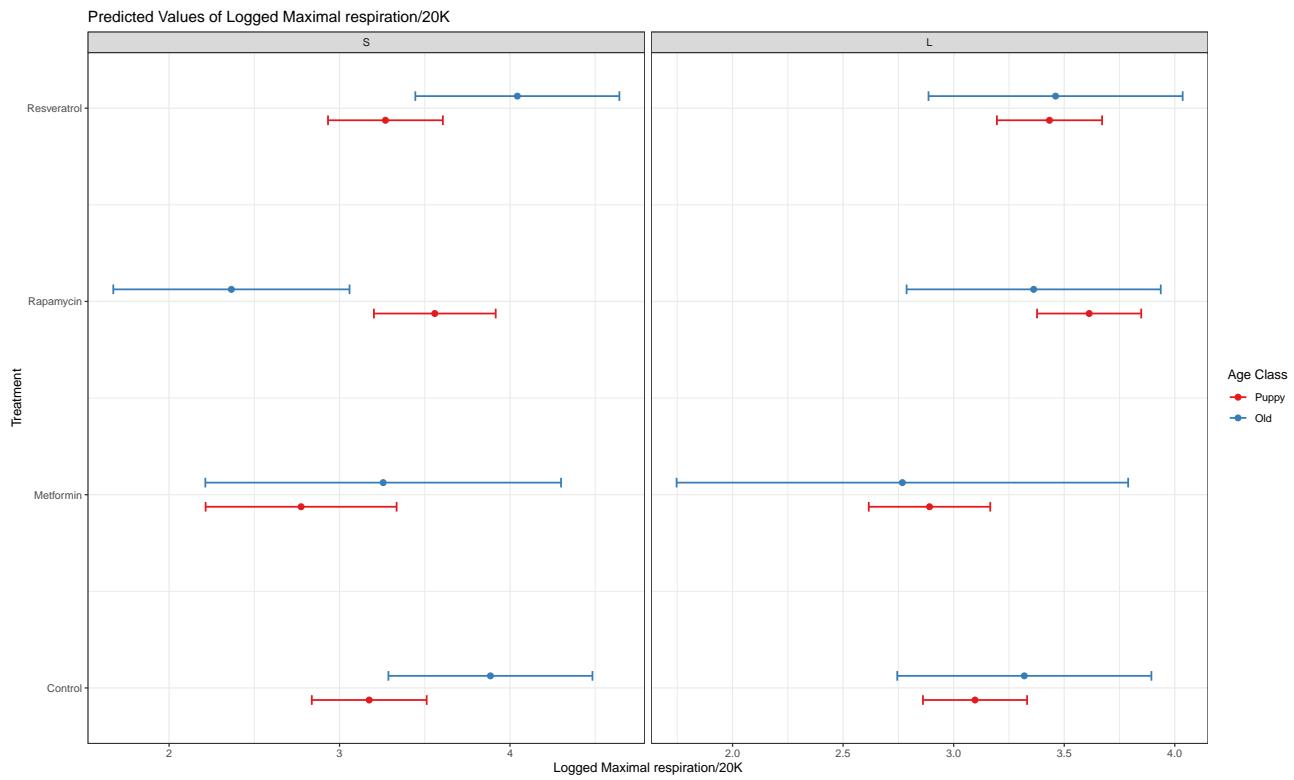


Figure 22: Marginal means for the full log transformed model for Maximal respiration/20K.

	Treatment	Age Class	Size Class	Est.	Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	3.1742		0.1719	306.9583	2.8360	3.5124
2	Metformin	Puppy	S	2.7746		0.2862	427.7107	2.2121	3.3371
3	Rapamycin	Puppy	S	3.5588		0.1823	336.9440	3.2002	3.9174
4	Resveratrol	Puppy	S	3.2692		0.1719	306.9583	2.9310	3.6075
5	Control	Old	S	3.8852		0.3054	282.6976	3.2841	4.4862
6	Metformin	Old	S	3.2561		0.5331	428.7225	2.2083	4.3040
7	Rapamycin	Old	S	2.3656		0.3537	356.8728	1.6700	3.0613
8	Resveratrol	Old	S	4.0432		0.3054	282.6976	3.4421	4.6442
9	Control	Puppy	L	3.0966		0.1202	293.4136	2.8600	3.3331
10	Metformin	Puppy	L	2.8908		0.1402	375.1970	2.6150	3.1665
11	Rapamycin	Puppy	L	3.6127		0.1202	293.4136	3.3761	3.8492
12	Resveratrol	Puppy	L	3.4333		0.1214	298.5435	3.1945	3.6721
13	Control	Old	L	3.3195		0.2932	299.7206	2.7425	3.8965
14	Metformin	Old	L	2.7680		0.5217	426.0204	1.7425	3.7934
15	Rapamycin	Old	L	3.3618		0.2932	299.7206	2.7848	3.9389
16	Resveratrol	Old	L	3.4609		0.2932	299.7206	2.8839	4.0379

Table 42: Summary of the marginal means for the full log transformed model for Maximal respiration/20k.

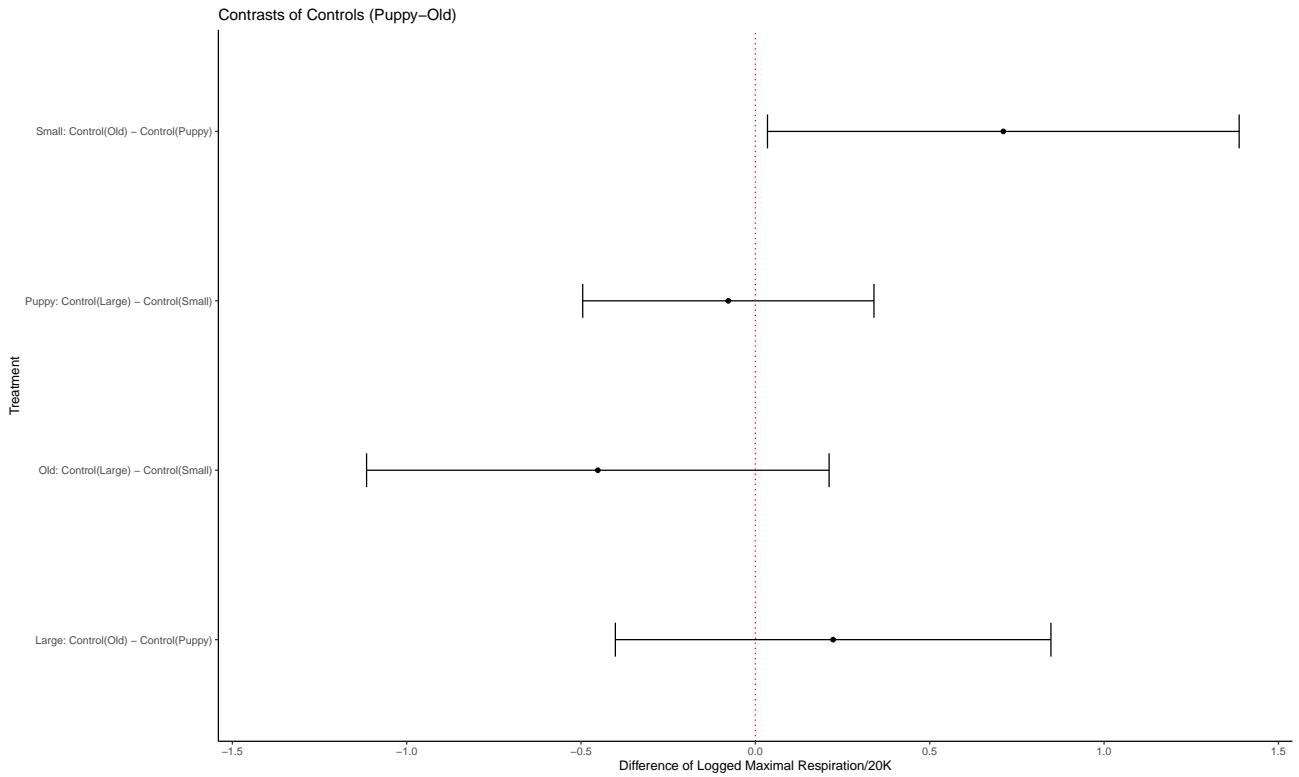


Figure 23: Pairwise treatment contrasts for the full log transformed model for Maximal respiration/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	0.2229	0.3174	298.2592	0.7024	0.6439	-0.4016	0.8475
2	Small: Control(Old) - Control(Puppy)	0.7110	0.3437	297.0934	2.0688	0.1577	0.0347	1.3873
3	Puppy: Control(Large) - Control(Small)	-0.0776	0.2122	297.1161	-0.3658	0.7148	-0.4953	0.3400
4	Old: Control(Large) - Control(Small)	-0.4519	0.3369	274.4248	-1.3413	0.3619	-1.1150	0.2113

Table 43: Summary of the pairwise control contrast for the full log transformed model for Maximal respiration/20k.

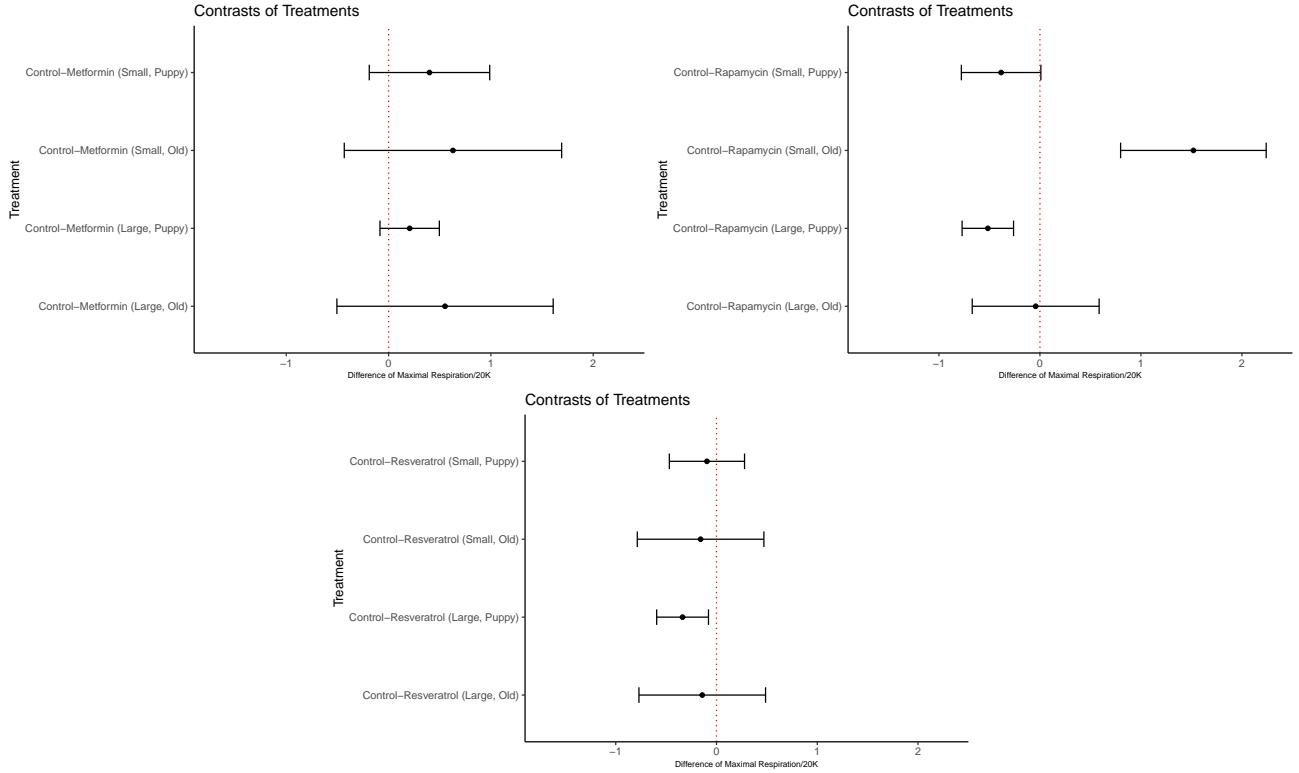


Figure 24: Pairwise treatment contrasts for the full log transformed model for Maximal respiration/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control-Metformin (Small, Puppy)	0.3996	0.2992	337.6067	1.3354	0.3653	-0.1890	0.9882
2	Control-Metformin (Large, Puppy)	0.2058	0.1475	318.4261	1.3953	0.3653	-0.0844	0.4960
3	Control-Metformin (Small, Old)	0.6290	0.5401	350.1486	1.1646	0.4199	-0.4333	1.6914
4	Control-Metformin (Large, Old)	0.5515	0.5373	343.3537	1.0265	0.4581	-0.5053	1.6083
5	Control-Rapamycin (Small, Puppy)	-0.3846	0.2000	314.7629	-1.9225	0.1663	-0.7782	0.0090
6	Control-Rapamycin (Large, Puppy)	-0.5161	0.1295	306.6566	-3.9867	0.0005	-0.7708	-0.2614
7	Control-Rapamycin (Small, Old)	1.5195	0.3664	325.8514	4.1467	0.0005	0.7986	2.2404
8	Control-Rapamycin (Large, Old)	-0.0424	0.3193	306.6566	-0.1326	0.8946	-0.6706	0.5859
9	Control-Resveratrol (Small, Puppy)	-0.0951	0.1897	306.6566	-0.5011	0.7179	-0.4683	0.2782
10	Control-Resveratrol (Large, Puppy)	-0.3367	0.1306	308.2037	-2.5781	0.0416	-0.5938	-0.0797
11	Control-Resveratrol (Small, Old)	-0.1580	0.3193	306.6566	-0.4948	0.7179	-0.7863	0.4703
12	Control-Resveratrol (Large, Old)	-0.1414	0.3193	306.6566	-0.4429	0.7179	-0.7697	0.4869

Table 44: Summary of the pairwise treatment contrasts for the full log transformed model for Maximal respiration/20K.

7.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	1.8916	0.3909	129.0209	4.8393	<0.0001
SexM	-0.1922	0.1328	117.2169	-1.4471	0.1505
breed.lifespan	0.1330	0.0340	125.2226	3.9063	0.0005
treatmentMetformin	-0.3148	0.1279	327.1866	-2.4618	0.0214
treatmentRapamycin	0.2928	0.1027	310.4836	2.8518	0.0093
treatmentResveratrol	0.2402	0.1008	307.7134	2.3824	0.0214

Table 45: Summary of the log-transformed best subsets model for Maximal respiration/20K.

7.3 Analysis

A log transform of Maximal respiration/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that breed lifespan and treatment with all three drugs (Metformin, Rapamycin and Resveratrol) are significantly associated with Maximal respiration/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 1.8916 - 0.1922X_{1i} + 0.1330X_{2i} - 0.3148X_{3i} + 0.2928X_{4i} + 0.2402X_{5i} + \epsilon_i.$$

Where

Y_i = Maximal respiration/20K

α_i = The random intercept for dog i

X_{1i} = Sex (1=Male, 0=Female)

X_{2i} = breed lifespan (in years)

X_{3i} = treatment group (1= Metformin, 0= Other)

X_{4i} = treatment group (1= Rapamycin, 0= Other)

X_{5i} = treatment group (1= Resveratrol, 0= Other)

for each observation $i = 1, 2, \dots, n$.

- We expect a 17.49% decrease in Maximal respiration/20K for male dogs compared to female dogs

- For every increase in breed lifespan by an year, we expect a 14.22% increase in Maximal respiration/20K.
- We expect a 27.01% decrease in Maximal respiration/20K for dogs treated with Metformin drug compared to dogs in control group or those treated with another drug
- We expect a 34.02% increase in Maximal respiration/20K for dogs treated with Rapamycin drug compared to dogs in control group or those treated with another drug
- We expect a 27.15% increase in Maximal respiration/20K for dogs treated with Resveratrol drug compared to dogs in control group or those treated with another drug

8 Spare Respiratory Capacity/20K

8.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	1.9029	0.4397	144.6140	4.3280	0.0005
Age.ClassOld	0.6773	0.3134	286.6423	2.1611	0.1538
SexM	-0.1637	0.1257	117.5288	-1.3020	0.3518
Size.ClassL	-0.1963	0.1982	296.5743	-0.9906	0.4167
breed.lifespan	0.0769	0.0359	128.4994	2.1409	0.1538
treatmentMetformin	-0.4334	0.2763	304.0886	-1.5683	0.3031
treatmentRapamycin	0.1951	0.1975	294.7290	0.9878	0.4167
treatmentResveratrol	0.1564	0.1900	288.3755	0.8235	0.4623
Age.ClassOld:treatmentMetformin	-0.6402	0.5649	310.2185	-1.1333	0.4167
Age.ClassOld:treatmentRapamycin	-0.9348	0.3887	290.3738	-2.4050	0.1512
Age.ClassOld:treatmentResveratrol	-0.3497	0.3484	275.8171	-1.0039	0.4167
Size.ClassL:treatmentMetformin	0.2565	0.3087	302.2797	0.8309	0.4623
Size.ClassL:treatmentRapamycin	0.0677	0.2339	289.8496	0.2895	0.8178
Size.ClassL:treatmentResveratrol	-0.0223	0.2261	284.9511	-0.0988	0.9214
Age.ClassOld:Size.ClassL	-0.7683	0.4236	300.3284	-1.8140	0.2120
Age.ClassOld:Size.ClassL:treatmentMetformin	1.0913	0.8384	323.2339	1.3017	0.3518
Age.ClassOld:Size.ClassL:treatmentRapamycin	1.0113	0.5183	289.4385	1.9511	0.1872
Age.ClassOld:Size.ClassL:treatmentResveratrol	0.6740	0.4881	281.8798	1.3807	0.3518

Table 46: Summary of the log-transformed full model for Spare Respiratory Capacity/20K.

8.1.1 Post-hoc Analysis

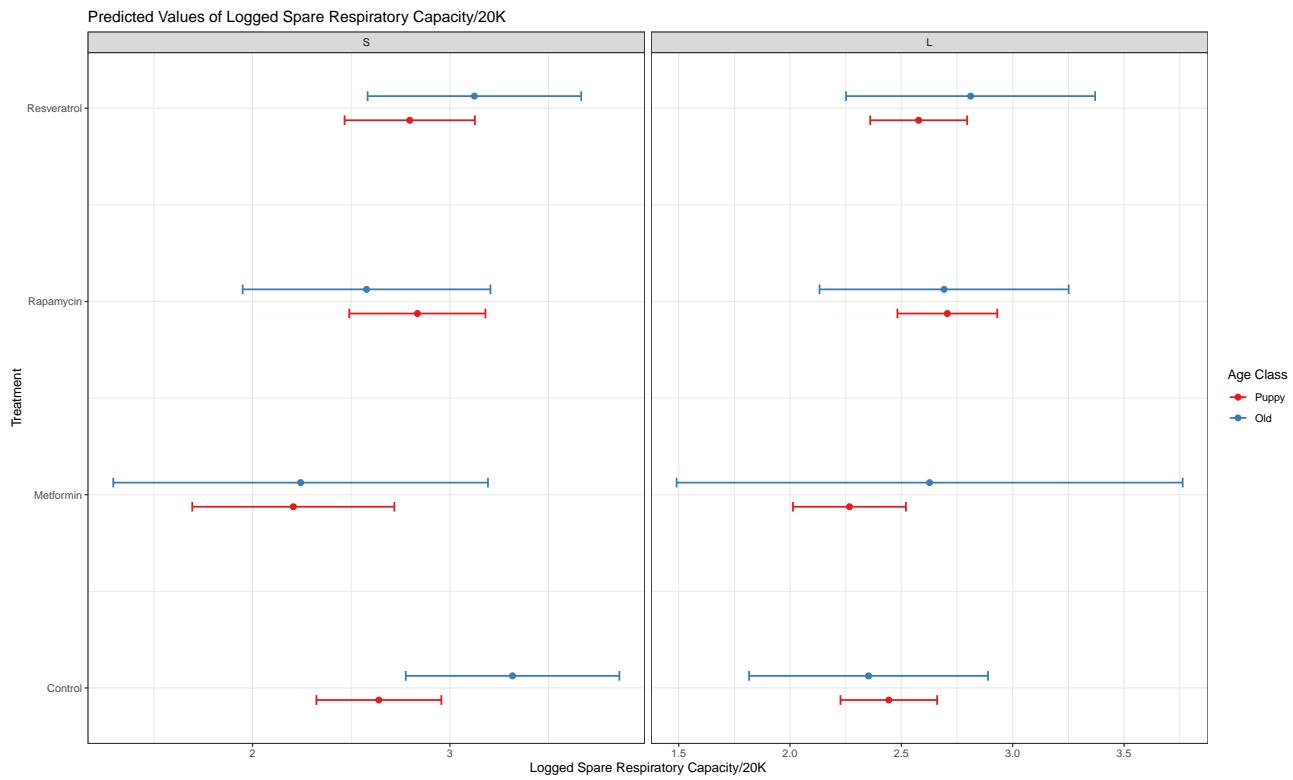


Figure 25: Marginal means for the full log transformed model for Spare Respiratory Capacity/20K.

	Treatment	Age Class	Size Class	Est.	Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	2.6400		0.1616	308.2641	2.3221	2.9579
2	Metformin	Puppy	S	2.2066		0.2616	391.2672	1.6923	2.7209
3	Rapamycin	Puppy	S	2.8351		0.1761	339.5712	2.4888	3.1814
4	Resveratrol	Puppy	S	2.7964		0.1686	324.4485	2.4648	3.1281
5	Control	Old	S	3.3172		0.2761	271.3692	2.7737	3.8608
6	Metformin	Old	S	2.2436		0.4851	391.5335	1.2900	3.1973
7	Rapamycin	Old	S	2.5776		0.3205	337.8385	1.9471	3.2081
8	Resveratrol	Old	S	3.1240		0.2761	271.3692	2.5804	3.6675
9	Control	Puppy	L	2.4437		0.1108	290.9520	2.2256	2.6617
10	Metformin	Puppy	L	2.2668		0.1295	357.9069	2.0122	2.5215
11	Rapamycin	Puppy	L	2.7065		0.1145	305.5786	2.4813	2.9317
12	Resveratrol	Puppy	L	2.5778		0.1111	290.0704	2.3592	2.7963
13	Control	Old	L	2.3526		0.2737	303.2684	1.8140	2.8913
14	Metformin	Old	L	2.6268		0.5811	381.2505	1.4843	3.7693
15	Rapamycin	Old	L	2.6920		0.2856	315.4006	2.1301	3.2538
16	Resveratrol	Old	L	2.8110		0.2854	315.2034	2.2493	3.3726

Table 47: Summary of the marginal means for the full log transformed model for Spare Respiratory Capacity/20k.

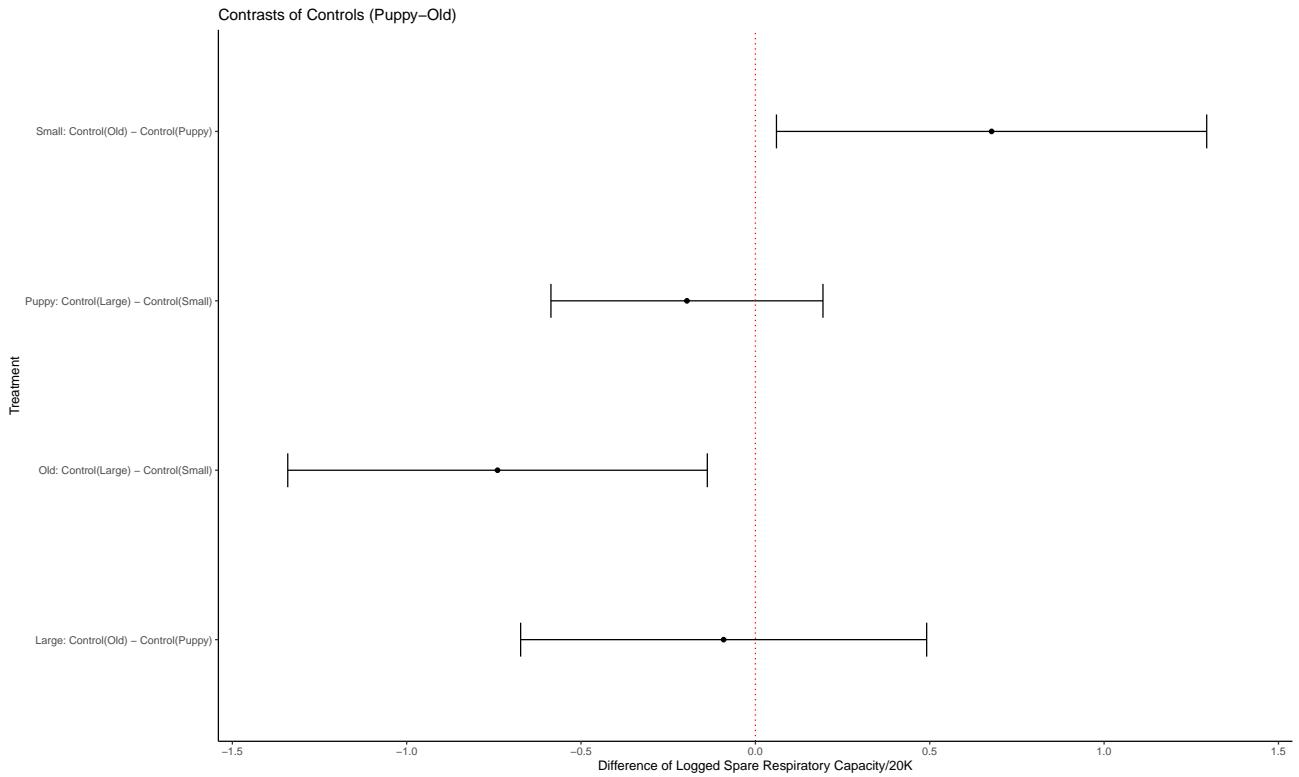


Figure 26: Pairwise treatment contrasts for the full log transformed model for Spare Respiratory Capacity/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	-0.0910	0.2958	301.3144	-0.3078	0.7585	-0.6732	0.4911
2	Small: Control(Old) - Control(Puppy)	0.6773	0.3134	288.1270	2.1609	0.0631	0.0604	1.2942
3	Puppy: Control(Large) - Control(Small)	-0.1963	0.1982	297.9664	-0.9904	0.4304	-0.5864	0.1938
4	Old: Control(Large) - Control(Small)	-0.7395	0.3055	264.3640	-2.4203	0.0631	-1.3411	-0.1379

Table 48: Summary of the pairwise control contrast for the full log transformed model for Spare Respiratory Capacity/20k.

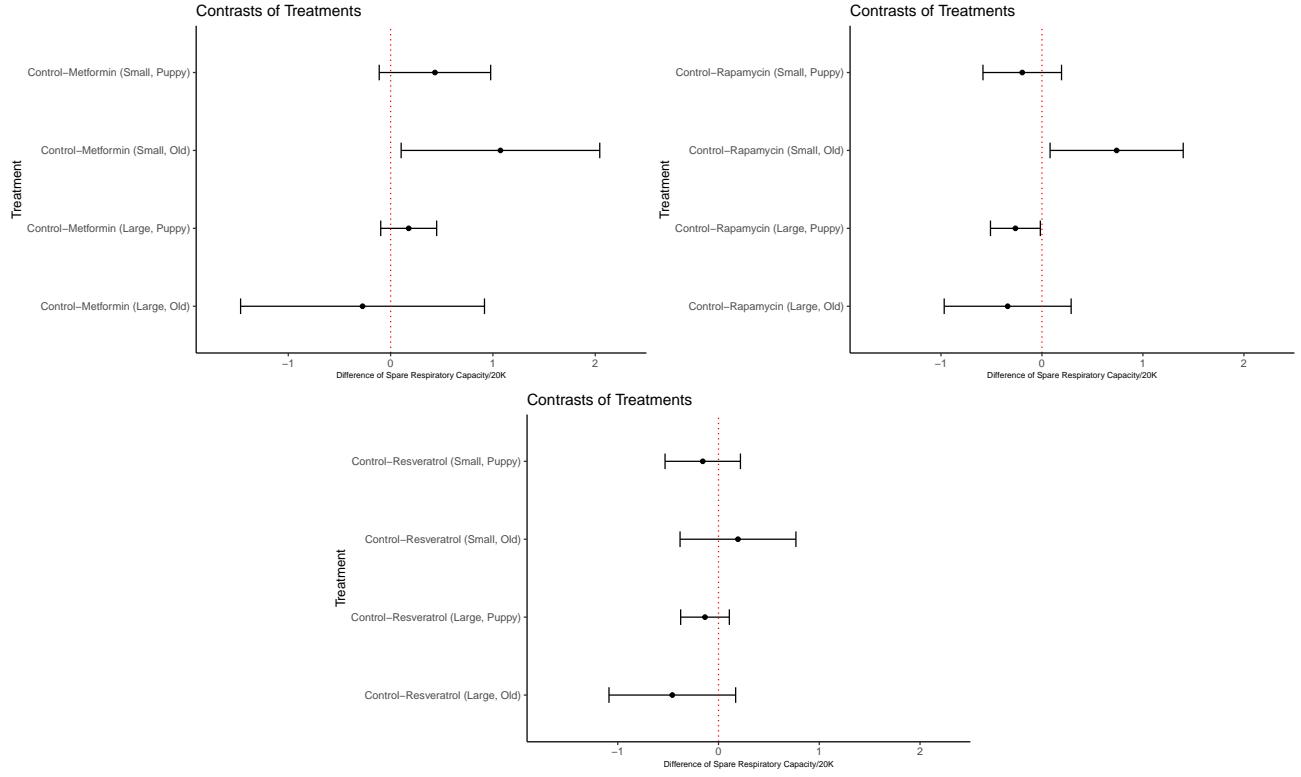


Figure 27: Pairwise treatment contrasts for the full log transformed model for Spare Respiratory Capacity/20K.

	Treatment Difference	estimate	SE	df	t.ratio	p.value	lower.CL	upper.CL
1	Control-Metformin (Small, Puppy)	0.4334	0.2767	305.4042	1.5663	0.3550	-0.1111	0.9778
2	Control-Metformin (Large, Puppy)	0.1768	0.1388	288.1982	1.2738	0.4075	-0.0964	0.4501
3	Control-Metformin (Small, Old)	1.0736	0.4932	314.0960	2.1766	0.1478	0.1031	2.0441
4	Control-Metformin (Large, Old)	-0.2742	0.6058	336.0447	-0.4526	0.6511	-1.4659	0.9175
5	Control-Rapamycin (Small, Puppy)	-0.1951	0.1977	296.1391	-0.9871	0.4326	-0.5841	0.1939
6	Control-Rapamycin (Large, Puppy)	-0.2628	0.1254	279.2546	-2.0963	0.1478	-0.5096	-0.0160
7	Control-Rapamycin (Small, Old)	0.7397	0.3349	290.4102	2.2086	0.1478	0.0805	1.3988
8	Control-Rapamycin (Large, Old)	-0.3393	0.3193	291.3607	-1.0626	0.4326	-0.9679	0.2892
9	Control-Resveratrol (Small, Puppy)	-0.1564	0.1901	289.8447	-0.8230	0.4934	-0.5306	0.2177
10	Control-Resveratrol (Large, Puppy)	-0.1341	0.1227	278.2436	-1.0927	0.4326	-0.3757	0.1075
11	Control-Resveratrol (Small, Old)	0.1933	0.2920	272.1642	0.6620	0.5548	-0.3816	0.7682
12	Control-Resveratrol (Large, Old)	-0.4583	0.3193	291.3639	-1.4353	0.3655	-1.0869	0.1702

Table 49: Summary of the pairwise treatment contrasts for the full log transformed model for Spare Respiratory Capacity/20K.

8.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	1.4687	0.3543	121.2542	4.1448	0.0002
SexM	-0.2308	0.1234	121.4292	-1.8712	0.0637
breed.lifespan	0.1185	0.0313	125.2022	3.7800	0.0004

Table 50: Summary of the log-transformed best subsets model for Spare Respiratory Capacity/20K.

8.3 Analysis

A log transform of Spare Respiratory Capacity/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that breed lifespan is significantly associated with Spare Respiratory Capacity/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 1.4687 - 0.2308X_{1i} + 0.1185X_{2i} + \epsilon_i.$$

Where

$$Y_i = \text{Spare Respiratory Capacity/20K}$$

$$\alpha_i = \text{The random intercept for dog } i$$

$$X_{1i} = \text{Sex (1=Male, 0=Female)}$$

$$X_{2i} = \text{breed lifespan (in years)}$$

for each observation $i = 1, 2, \dots, n$.

- We expect a 20.61% decrease in Spare Respiratory Capacity/20K for male dogs compared to female dogs
- For every increase in breed lifespan by an year, we expect a 12.58% increase in Spare Respiratory Capacity/20K.

9 Non-mito Respiration/20K

9.1 Full Transformed Model

	Estimate	SE	DF	t	p
(Intercept)	1.3498	0.3469	137.4260	3.8917	0.0014
Age.ClassOld	0.6325	0.2441	270.6566	2.5915	0.0363
SexM	-0.0759	0.1010	117.5690	-0.7519	0.7422
Size.ClassL	-0.3028	0.1507	270.6963	-2.0093	0.1365
breed.lifespan	0.0823	0.0286	125.9781	2.8739	0.0214
treatmentMetformin	-0.1186	0.2037	328.8222	-0.5823	0.7765
treatmentRapamycin	0.5648	0.1359	308.8477	4.1547	0.0008
treatmentResveratrol	0.2362	0.1288	301.4007	1.8337	0.1741
Age.ClassOld:treatmentMetformin	0.2802	0.4208	337.3246	0.6660	0.7588
Age.ClassOld:treatmentRapamycin	-0.8566	0.2838	316.7360	-3.0179	0.0165
Age.ClassOld:treatmentResveratrol	-0.0415	0.2522	301.4007	-0.1648	0.8816
Size.ClassL:treatmentMetformin	0.2715	0.2268	326.9214	1.1973	0.4641
Size.ClassL:treatmentRapamycin	0.0673	0.1619	306.6470	0.4158	0.8134
Size.ClassL:treatmentResveratrol	0.0234	0.1566	302.0942	0.1491	0.8816
Age.ClassOld:Size.ClassL	-0.1015	0.3260	278.8814	-0.3114	0.8502
Age.ClassOld:Size.ClassL:treatmentMetformin	-0.4987	0.5664	335.5043	-0.8805	0.6826
Age.ClassOld:Size.ClassL:treatmentRapamycin	0.5384	0.3678	310.5149	1.4637	0.3246
Age.ClassOld:Size.ClassL:treatmentResveratrol	-0.1708	0.3443	301.5442	-0.4962	0.7973

Table 51: Summary of the log-transformed full model for Non-mito respiration/20K.

9.1.1 Post-hoc Analysis

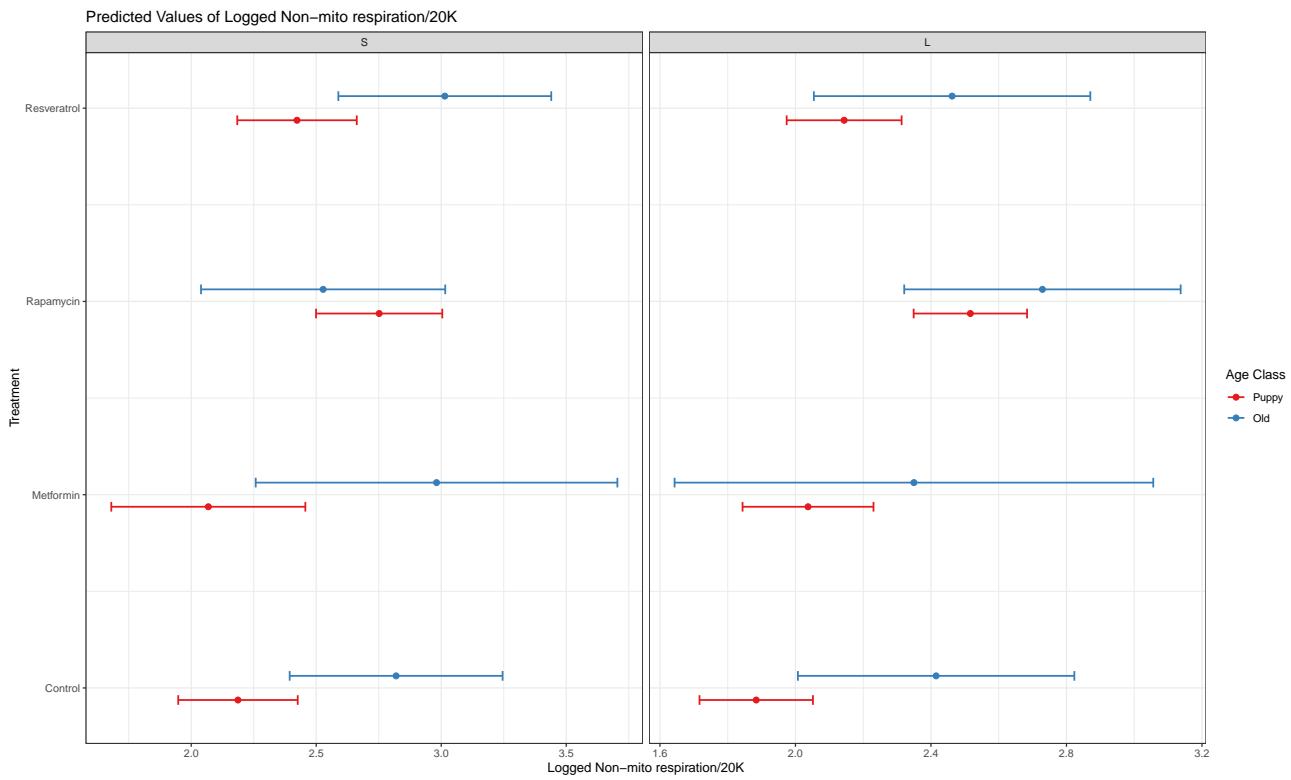


Figure 28: Marginal means for the full log transformed model for Non-mito respiration/20K.

	Treatment	Age Class	Size Class	Est.	Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	Puppy	S	2.1870		0.1219	284.5394	1.9471	2.4270
2	Metformin	Puppy	S	2.0685		0.1982	427.7787	1.6789	2.4580
3	Rapamycin	Puppy	S	2.7518		0.1288	315.5796	2.4983	3.0053
4	Resveratrol	Puppy	S	2.4232		0.1219	284.5394	2.1832	2.6632
5	Control	Old	S	2.8196		0.2172	262.0428	2.3918	3.2474
6	Metformin	Old	S	2.9812		0.3695	427.9999	2.2550	3.7075
7	Rapamycin	Old	S	2.5277		0.2493	338.4996	2.0373	3.0181
8	Resveratrol	Old	S	3.0142		0.2172	262.0428	2.5865	3.4420
9	Control	Puppy	L	1.8842		0.0854	271.8428	1.7160	2.0524
10	Metformin	Puppy	L	2.0371		0.0986	356.2045	1.8431	2.2311
11	Rapamycin	Puppy	L	2.5163		0.0854	271.8428	2.3481	2.6844
12	Resveratrol	Puppy	L	2.1437		0.0866	279.5213	1.9733	2.3142
13	Control	Old	L	2.4152		0.2082	277.7249	2.0054	2.8250
14	Metformin	Old	L	2.3497		0.3609	426.5088	1.6403	3.0591
15	Rapamycin	Old	L	2.7291		0.2082	277.7249	2.3193	3.1389
16	Resveratrol	Old	L	2.4624		0.2082	277.7249	2.0526	2.8722

Table 52: Summary of the marginal means for the full log transformed model for Non-mito respiration/20k.

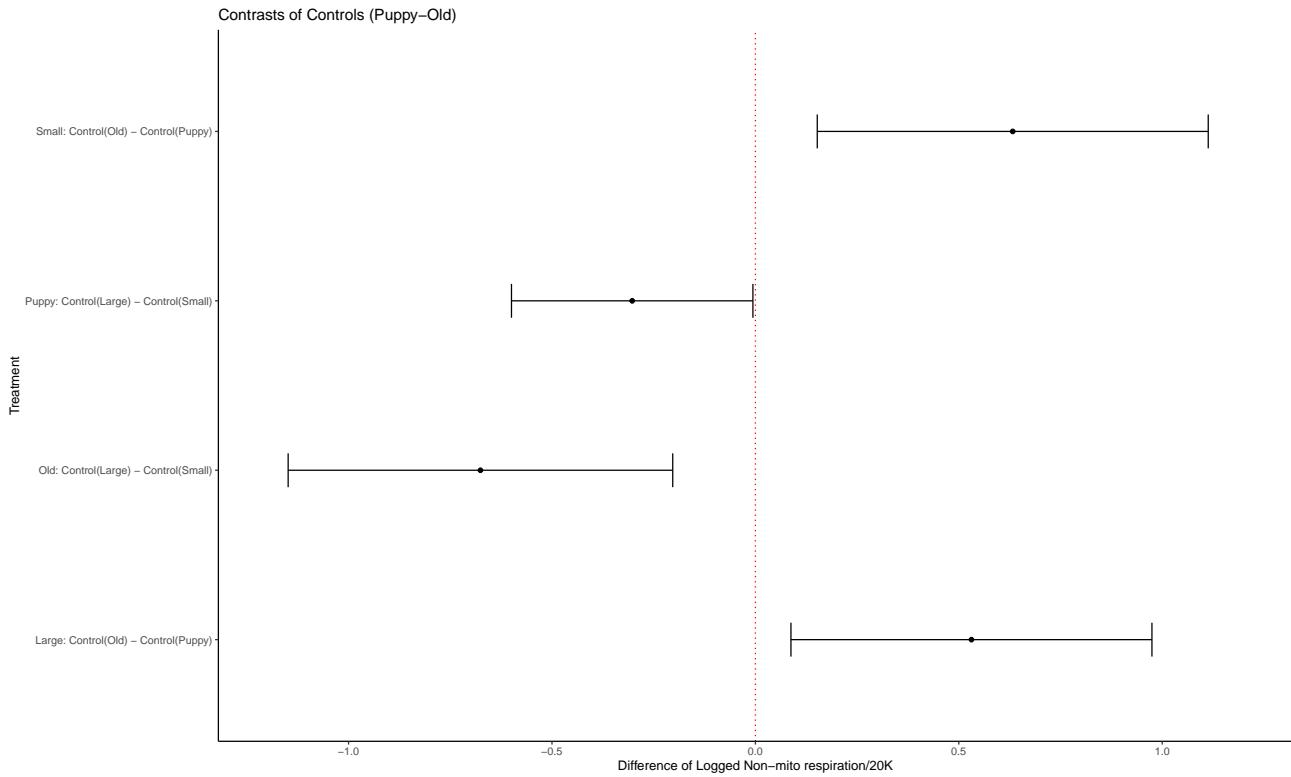


Figure 29: Pairwise treatment contrasts for the full log transformed model for Non-mito respiration/20K.

	Control Difference	Estimate	SE	DF	t ratio	p	CL Lower	CL Upper
1	Large: Control(Old) - Control(Puppy)	0.5310	0.2254	276.3573	2.3562	0.0255	0.0874	0.9747
2	Small: Control(Old) - Control(Puppy)	0.6325	0.2441	275.2707	2.5914	0.0201	0.1520	1.1131
3	Puppy: Control(Large) - Control(Small)	-0.3028	0.1507	275.3099	-2.0093	0.0455	-0.5996	-0.0061
4	Old: Control(Large) - Control(Small)	-0.6759	0.2400	254.6985	-2.8159	0.0201	-1.1485	-0.2032

Table 53: Summary of the pairwise control contrast for the full log transformed model for Non-mito respiration/20k.

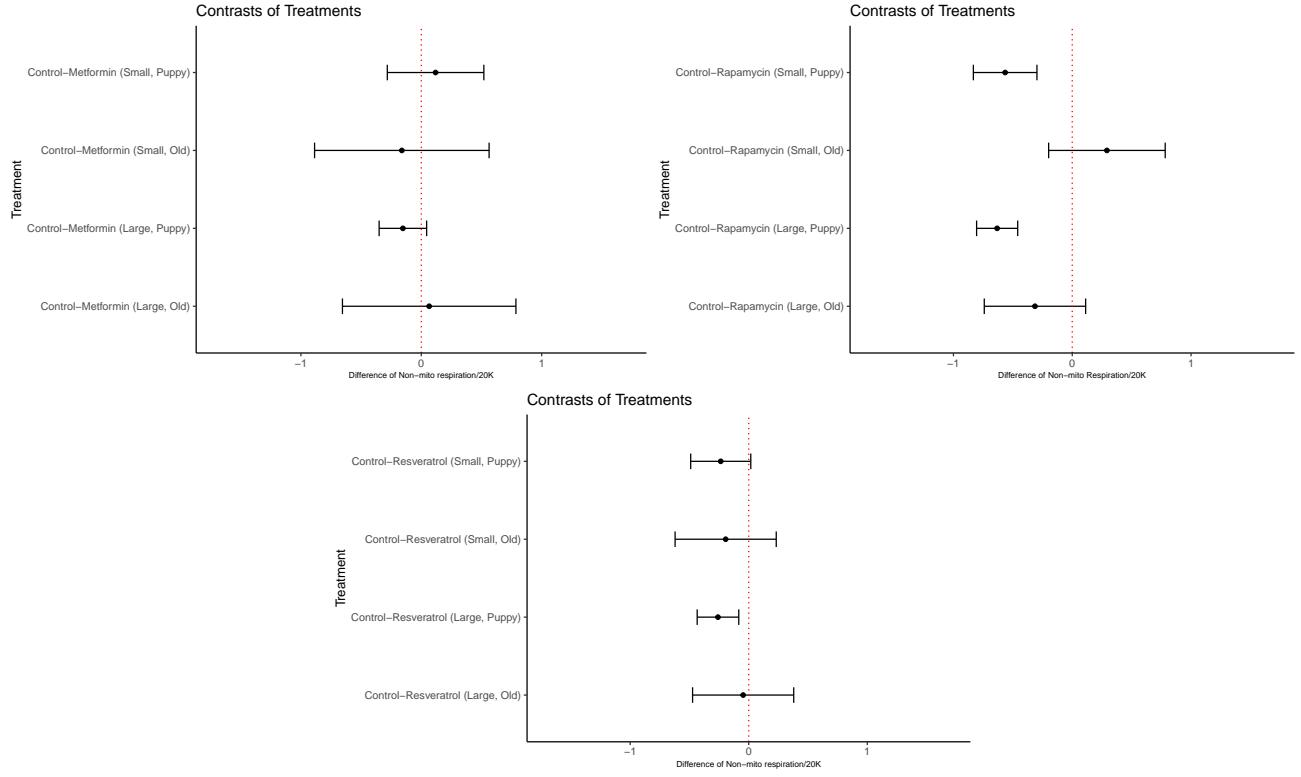


Figure 30: Pairwise treatment contrasts for the full log transformed model for Non-mito respiration/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control-Metformin (Small, Puppy)	0.1186	0.2039	332.3335	0.5817	0.7482	-0.2824	0.5196
2	Control-Metformin (Large, Puppy)	-0.1529	0.1003	316.0062	-1.5246	0.2975	-0.3502	0.0444
3	Control-Metformin (Small, Old)	-0.1617	0.3685	343.7036	-0.4387	0.7934	-0.8865	0.5632
4	Control-Metformin (Large, Old)	0.0655	0.3663	337.2336	0.1789	0.8581	-0.6550	0.7860
5	Control-Rapamycin (Small, Puppy)	-0.5648	0.1360	312.8184	-4.1538	0.0003	-0.8323	-0.2972
6	Control-Rapamycin (Large, Puppy)	-0.6321	0.0879	305.5212	-7.1905	<0.0001	-0.8050	-0.4591
7	Control-Rapamycin (Small, Old)	0.2919	0.2494	322.7005	1.1705	0.4160	-0.1987	0.7824
8	Control-Rapamycin (Large, Old)	-0.3139	0.2168	305.5212	-1.4476	0.2975	-0.7405	0.1128
9	Control-Resveratrol (Small, Puppy)	-0.2362	0.1288	305.5212	-1.8337	0.2030	-0.4896	0.0173
10	Control-Resveratrol (Large, Puppy)	-0.2595	0.0891	307.6229	-2.9119	0.0154	-0.4349	-0.0842
11	Control-Resveratrol (Small, Old)	-0.1946	0.2168	305.5212	-0.8977	0.5551	-0.6213	0.2320
12	Control-Resveratrol (Large, Old)	-0.0472	0.2168	305.5212	-0.2175	0.8581	-0.4738	0.3795

Table 54: Summary of the pairwise treatment contrasts for the full log transformed model for Non-mito respiration/20K.

9.2 Best Subsets Model

	Estimate	SE	DF	t	p
(Intercept)	0.7026	0.3004	129.7161	2.3387	0.0313
SexM	-0.1358	0.1027	121.1014	-1.3219	0.2264
breed.lifespan	0.1372	0.0262	127.0531	5.2319	<0.0001
treatmentMetformin	0.0713	0.0848	324.4813	0.8407	0.4011
treatmentRapamycin	0.5201	0.0679	312.4017	7.6619	<0.0001
treatmentResveratrol	0.2289	0.0668	310.6318	3.4284	0.0014

Table 55: Summary of the log-transformed best subsets model for Non-mito respiration/20K.

9.2.1 Post-hoc Analysis

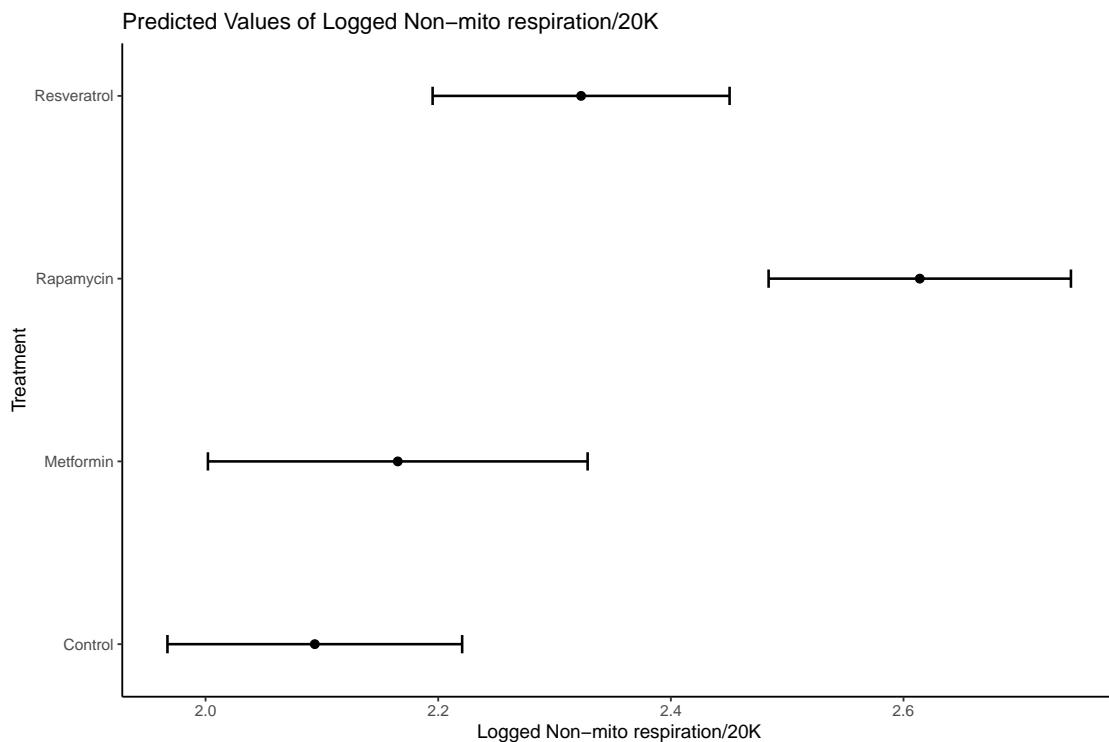


Figure 31: Marginal means for the log-transformed best subsets model for Non-mito respiration/20K.

	Treatment	Est. Marg. Mean	SE	DF	CI Lower	CI Upper
1	Control	2.0939	0.0646	277.1930	1.9667	2.2211
2	Metformin	2.1652	0.0833	411.4607	2.0015	2.3290
3	Rapamycin	2.6140	0.0663	293.2499	2.4835	2.7445
4	Resveratrol	2.3228	0.0651	281.8749	2.1946	2.4511

Table 56: Summary of the marginal means for the log-transformed best subsets model for Non-mito respiration/20K.

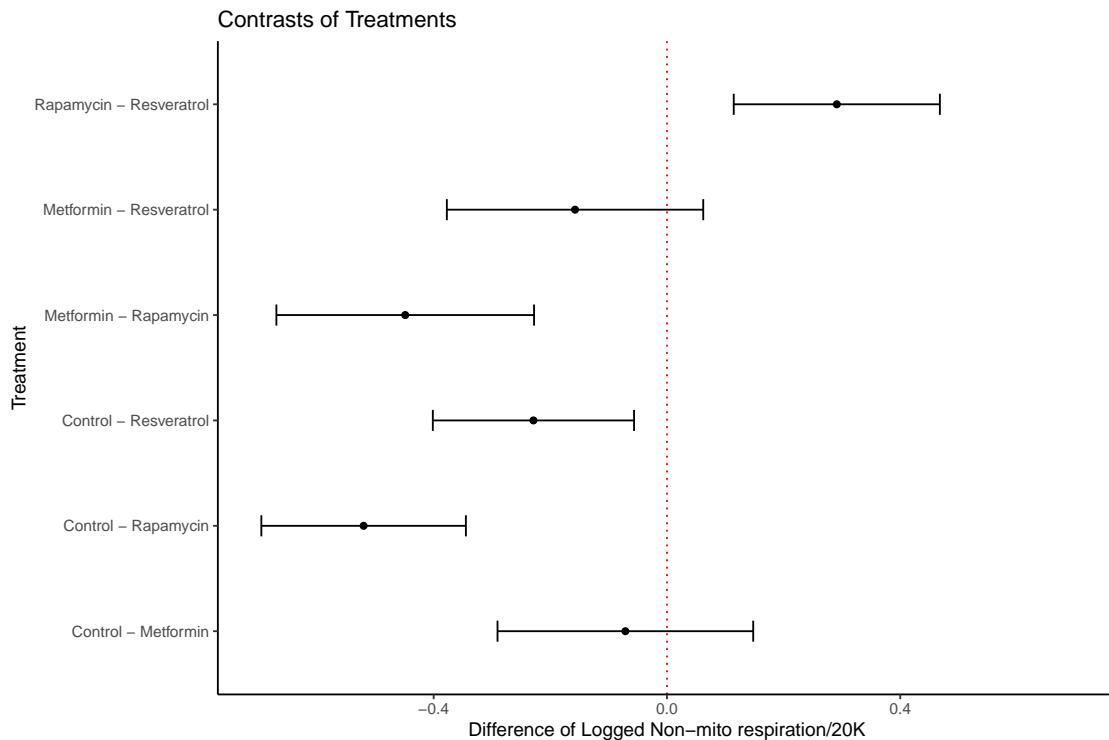


Figure 32: Pairwise treatment contrasts for the log-transformed best subsets model for Non-mito Respiration/20K.

	Treatment Difference	Estimate	SE	DF	t ratio	p	CI Lower	CI Upper
1	Control - Metformin	-0.0713	0.0849	329.0798	-0.8403	0.8352	-0.2905	0.1479
2	Control - Rapamycin	-0.5201	0.0679	317.2991	-7.6613	<0.0001	-0.6954	-0.3448
3	Control - Resveratrol	-0.2289	0.0668	315.5701	-3.4283	0.0038	-0.4014	-0.0565
4	Metformin - Rapamycin	-0.4488	0.0855	329.0940	-5.2463	<0.0001	-0.6696	-0.2279
5	Metformin - Resveratrol	-0.1576	0.0851	328.0449	-1.8517	0.2512	-0.3774	0.0622
6	Rapamycin - Resveratrol	0.2911	0.0684	318.4153	4.2551	0.0002	0.1144	0.4679

Table 57: Summary of the pairwise treatment contrasts for the log-transformed best subsets model for Non-mito respiration/20K.

9.3 Analysis

A log transform of Non-mito respiration/20K was completed to correct issues with the diagnostic plots in the full model. After completing model building from the full set of explanatory variables using best subsets, the reduced model that minimizes BIC suggests that breed lifespan and treatment with drugs Rapamycin and Resveratrol are significantly associated with Non-mito respiration/20K.

This yields a regression equation of,

$$\log(Y_i) = \alpha_i + 0.7026 - 0.1358X_{1i} + 0.1372X_{2i} + 0.0713X_{3i} + 0.5201X_{4i} + 0.2289X_{5i} + \epsilon_i.$$

Where

$$Y_i = \text{Non-mito respiration/20K}$$

$$\alpha_i = \text{The random intercept for dog } i$$

$$X_{1i} = \text{Sex (1=Male, 0=Female)}$$

$$X_{2i} = \text{breed lifespan (in years)}$$

$$X_{3i} = \text{treatment group (1= Metformin, 0= Other)}$$

$$X_{4i} = \text{treatment group (1= Rapamycin, 0= Other)}$$

$$X_{5i} = \text{treatment group (1= Resveratrol, 0= Other)}$$

for each observation $i = 1, 2, \dots, n$.

- We expect a 12.7% decrease in Non-mito respiration/20K for male dogs compared to female dogs

- For every increase in breed lifespan by an year, we expect a 14.71% increase in Non-mito respiration/20K.
- We expect a 7.39% increase in Non-mito respiration/20K for dogs treated with Metformin drug compared to dogs in control group or those treated with another drug
- We expect a 68.22% increase in Non-mito respiration/20K for dogs treated with Rapamycin drug compared to dogs in control group or those treated with another drug
- We expect a 25.72% increase in Non-mito respiration/20K for dogs treated with Resveratrol drug compared to dogs in control group or those treated with another drug

Variable (units)	Size and Age	Treatment	Mean \pm SEM	Treatment	Mean \pm SEM	Treatment	Mean \pm SEM	Treatment	Mean \pm SEM
Basal OCR (pmole/min/20K cells)	Large old	Control	31.06 \pm 12.91	Metformin	13.14 \pm 7.47	Rapamycin	33.86 \pm 8.20	Resveratrol	24.55 \pm 2.83
Basal OCR (pmole/min/20K cells)	Large puppy	Control	14.90 \pm 1.52	Metformin	11.59 \pm 1.28	Rapamycin	26.88 \pm 2.90	Resveratrol	21.63 \pm 2.24
Basal OCR (pmole/min/20K cells)	Small old	Control	57.58 \pm 22.20	Metformin	41.15 \pm 28.18	Rapamycin	23.22 \pm 4.81	Resveratrol	56.28 \pm 12.90
Basal OCR (pmole/min/20K cells)	Small puppy	Control	25.84 \pm 5.72	Metformin	7.95 \pm 1.62	Rapamycin	30.22 \pm 5.30	Resveratrol	40.62 \pm 11.73
Proton leak (pmole/min/20K cells)	Large old	Control	10.77 \pm 5.11	Metformin	15.44 \pm 12.09	Rapamycin	20.65 \pm 7.63	Resveratrol	10.8 \pm 3.20
Proton leak (pmole/min/20K cells)	Large puppy	Control	6.67 \pm 1.38	Metformin	6.04 \pm 1.44	Rapamycin	12.54 \pm 2.37	Resveratrol	7.12 \pm 0.85
Proton leak (pmole/min/20K cells)	Small old	Control	18.23 \pm 4.94	Metformin	45.69 \pm 29.25	Rapamycin	13.61 \pm 3.45	Resveratrol	26.78 \pm 6.06
Proton leak (pmole/min/20K cells)	Small puppy	Control	10.65 \pm 3.13	Metformin	4 \pm 0.67	Rapamycin	16.44 \pm 4.66	Resveratrol	12.42 \pm 2.81
Maximal respiration (pmole/min/20K cells)	Large old	Control	43.15 \pm 12.55	Metformin	24.33 \pm 14.35	Rapamycin	55.96 \pm 12.92	Resveratrol	40.2 \pm 4.28
Maximal respiration (pmole/min/20K cells)	Large puppy	Control	26.86 \pm 2.33	Metformin	22.12 \pm 2.10	Rapamycin	45.51 \pm 5.45	Resveratrol	35.16 \pm 2.94
Maximal respiration (pmole/min/20K cells)	Small old	Control	110.83 \pm 42.25	Metformin	52.53 \pm 26.09	Rapamycin	39.68 \pm 8.13	Resveratrol	99.7 \pm 22.72
Maximal respiration (pmole/min/20K cells)	Small puppy	Control	48.85 \pm 11.15	Metformin	14.63 \pm 2.02	Rapamycin	45.7 \pm 8.42	Resveratrol	65.71 \pm 18.94
Spare respiratory capacity (pmole/min/20K cells)	Large old	Control	13.04 \pm 1.99	Metformin	15.62 \pm 9.03	Rapamycin	27.01 \pm 6.12	Resveratrol	18.36 \pm 2.05
Spare respiratory capacity (pmole/min/20K cells)	Large puppy	Control	12.70 \pm 1.13	Metformin	11.01 \pm 1.27	Rapamycin	22.71 \pm 3.66	Resveratrol	14.94 \pm 1.38
Spare respiratory capacity (pmole/min/20K cells)	Small old	Control	53.24 \pm 21.01	Metformin	11.38 \pm 4.02	Rapamycin	17.56 \pm 4.11	Resveratrol	43.41 \pm 11.10
Spare respiratory capacity (pmole/min/20K cells)	Small puppy	Control	25.02 \pm 5.85	Metformin	6.69 \pm 1.03	Rapamycin	20.53 \pm 4.79	Resveratrol	30.43 \pm 8.85
Non-mitochondrial respiration (pmole/min/20K cells)	Large old	Control	17.33 \pm 5.57	Metformin	14.63 \pm 8.49	Rapamycin	25.41 \pm 6.31	Resveratrol	13.46 \pm 1.60
Non-mitochondrial respiration (pmole/min/20K cells)	Large puppy	Control	7.77 \pm 1.04	Metformin	9.84 \pm 1.39	Rapamycin	16.93 \pm 2.66	Resveratrol	9.79 \pm 8.80
Non-mitochondrial respiration (pmole/min/20K cells)	Small old	Control	29.26 \pm 9.11	Metformin	35.85 \pm 19.71	Rapamycin	16.13 \pm 2.89	Resveratrol	31.89 \pm 7.11
Non-mitochondrial respiration (pmole/min/20K cells)	Small puppy	Control	12.85 \pm 2.26	Metformin	6.99 \pm 0.92	Rapamycin	19.10 \pm 3.62	Resveratrol	19.57 \pm 4.85
Non-mitochondrial respiration (pmole/min/20K cells)	Large old	Control	57.54 \pm 11.31	Metformin	69.49 \pm 15.72	Rapamycin	86.9 \pm 28.41	Resveratrol	62.45 \pm 17.21
Glycolysis (mpH/min/20k cells)	Large puppy	Control	46.04 \pm 6.06	Metformin	81.85 \pm 16.25	Rapamycin	57.41 \pm 9.55	Resveratrol	52.18 \pm 7.31
Glycolysis (mpH/min/20k cells)	Small old	Control	59.98 \pm 7.42	Metformin	138.25 \pm 54.92	Rapamycin	116.86 \pm 44.32	Resveratrol	67.82 \pm 8.26
Glycolysis (mpH/min/20k cells)	Small puppy	Control	45.22 \pm 4.12	Metformin	76.78 \pm 17.25	Rapamycin	68.66 \pm 11.40	Resveratrol	61.11 \pm 9.05
Glycolytic capacity (mpH/min/20k cells)	Large old	Control	69.72 \pm 13.42	Metformin	52.78 \pm 16.15	Rapamycin	87.7 \pm 27.21	Resveratrol	77.68 \pm 24.87
Glycolytic capacity (mpH/min/20k cells)	Large puppy	Control	51.01 \pm 5.38	Metformin	68.95 \pm 11.97	Rapamycin	65.68 \pm 9.27	Resveratrol	59.06 \pm 6.75
Glycolytic capacity (mpH/min/20k cells)	Small old	Control	73.80 \pm 9.22	Metformin	113.08 \pm 47.24	Rapamycin	157.82 \pm 68.29	Resveratrol	96.25 \pm 17.92
Glycolytic capacity (mpH/min/20k cells)	Small puppy	Control	46.98 \pm 4.61	Metformin	61.77 \pm 9.53	Rapamycin	67.92 \pm 10.94	Resveratrol	69.39 \pm 11.52
Non-glycolytic acidification (mpH/min/20k cells)	Large old	Control	20.27 \pm 3.44	Metformin	13.26 \pm 3.69	Rapamycin	27.7 \pm 7.57	Resveratrol	24.8 \pm 7.27
Non-glycolytic acidification (mpH/min/20k cells)	Large puppy	Control	10.75 \pm 0.99	Metformin	15.01 \pm 2.54	Rapamycin	19.23 \pm 3.63	Resveratrol	15.87 \pm 2.01
Non-glycolytic acidification (mpH/min/20k cells)	Small old	Control	27.89 \pm 4.51	Metformin	31.92 \pm 13.22	Rapamycin	47.68 \pm 21.23	Resveratrol	30.86 \pm 4.84
Non-glycolytic acidification (mpH/min/20k cells)	Small puppy	Control	13.24 \pm 1.54	Metformin	16.05 \pm 2.70	Rapamycin	22.07 \pm 3.54	Resveratrol	22.31 \pm 2.89

Table 0: Averages \pm SEM per each cellular metabolic rate variable within each group.

References

- Benjamini, Y. and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, 57(1):289–300.
- Dunn, O. J. (1961). Multiple comparisons among means. *Journal of the American Statistical Association*, 56(293):52–64.
- Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82(13):1–26.
- Morgan, J. and Tatar, J. (1972). Calculation of the residual sum of squares for all possible regressions. *Technometrics*, 14:317–325.
- R Core Team (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Schwarz, G. et al. (1978). Estimating the dimension of a model. *The annals of statistics*, 6(2):461–464.